DISSERTATION

UNDERSTANDING THE IMPACT OF CARCASS SIZE, CHILLING RATE, AND ELECTRICAL STIMULATION ON BEEF QUALITY

Submitted by

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ABSTRACT

UNDERSTANDING THE IMPACT OF CARCASS SIZE, CHILLING RATE, AND ELECTRICAL STIMULATION ON BEEF QUALITY

Increasing carcass sizes and mass make it difficult for packers to appropriately chill beef carcasses, resulting in issues associated with tenderness and color. The wide variability in carcass size and weight and the lack of management practices to address it represent a challenge that the industry must address. To our knowledge, few studies have looked at the combined impact of chilling and electrical stimulation on postmortem biochemistry, tenderness, juiciness and color among the current consist of US beef carcasses, hence justifying this study. The study was conducted in two major parts:

The first part focused on the effects of carcass size, chilling rate, and electrical stimulation on temperature and pH decline and postmortem biochemistry. Cattle (N =162, < 30 month) were randomly selected at two beef processing plants in the US. The left or right side of each carcass was electrically stimulated (ES) whereas the matching side was not electrically stimulated (NES). Matched sides were conventionally spray-chilled (CC) or delayed spray-chilled (DC). Deep tissue and surface temperature were continuously monitored during chilling in addition to temperature and pH measurements obtained from the muscles *Semimembranosus* (SM), *Longissimus lumborum* (LL), and *Psoas major* (PM) at an initial time (45 to 60 min), 6 h, 12 h, and final chilling time (18 to 28 h postmortem). A six-member panel evaluated the color of the tenderloin (PM). The L*, a*, and b* values of the PM were measured. A nonlinear regression model was fitted to the continuous deep and surface temperatures. Electrical stimulation improved ($P < 0.05$) the

tenderloin color of light weight carcasses but not ($P > 0.05$) that of heavy weight carcasses. Temperature decline was faster ($P < 0.05$) in the SM and LL of heavy weight and delay chilled carcasses while pH decline was slower ($P < 0.05$). The exponential decay models for deep and surface temperatures showed that the rate of cooling differed ($P < 0.05$) due to the combination of treatment factors. Heavy weight carcasses had slower rates of chilling ($P < 0.05$). Variability in carcass size resulted in differences in chilling rate.

In the second part of the study, the effects of the treatment factors on beef tenderness were determined. Steaks from the loins (LL) collected from the previous study were randomly assigned to 14, 21, 28, or 35 d aging periods. While sensory evaluation was performed on the 14 d steaks, all the aging groups were used to determine Warner-Braztler shear force (WBSF) and slice shear force (SSF) values. Results showed that sensory panel scores were not affected ($P > 0.05$) by treatment factors. However, WBSF and SSF were affected ($P < 0.05$) by carcass size and chilling rate. Aging curves were developed using an exponential decay model to predict aging response and describe the tenderization process for the treatments groups. The models indicated significant differences in the rate and extent of tenderization between different treatment groups.

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DEDICATION

This dissertation is dedicated to my mother Mrs Helene Doumassem and my father Mr. Ngarari Djimsa who have always believed in me and pushed me to always do my best! I hope I made you both proud!

TABLE OF CONTENTS

ABSTRACT ii

ACKNOWLEDGEMENTS iv

DEDICATION v

LIST OF TABLES ix

LIST OF FIGURES xiii

INTRODUCTION 1

CHAPTER I 3

REVIEW OF LITERATURE 3

Electrical stimulation 3

Effect of electrical stimulation on postmortem metabolism 4

The ES and prevention of cold shortening 8

Effects of electrical stimulation on structural changes 9

Effects of electrical stimulation on postmortem proteolytic activity 10

Differential response of muscles to ES 12

Effects of electrical stimulation on meat color 13

Types of electrical stimulation 14

Postmortem glycolysis 15

Biochemical changes during postmortem metabolism 16

Issues during postmortem metabolism 25

Effects of pre-harvest conditions on glycogen depletion 27

Tenderness and tenderization 31

Effects of carcass size on meat quality 35
REFERENCES 38
CHAPTER II 50
THE IMPACT OF CARCASS SIZE, RATE OF CHILLING, AND ELECTRICAL STIMULATION ON BEEF POSTMORTEM GLYCOLYSIS AND COLOR 50
Introduction 50
Material and methods 52
Statistical analysis 55
Results 57
Discussion 66
Conclusion 73
TABLES AND FIGURES 75
REFERENCES 93
CHAPTER III 100
UNDERSTANDING THE IMPACT OF CARCASS SIZE, RATE OF CHILLING, AND ELECTRICAL STIMULATION ON BEEF TENDERNESS 100
Material and methods 102
Statistical analysis 104
Results and discussion 105
Conclusion 113
TABLES AND FIGURES 115
REFERENCES 129
APPENDICES 150

LIST OF TABLES

Table 1: Main effects of transportation, facility location, and behavior measurements on exsanguination blood samples of glucose and lactate in fed-cattle at two geographically different commercial processing facilities (N=197) 75

Table 2: Effects of carcass size, chilling rate, and electrical stimulation on temperature decline in the Longissimus lumborum (LL), Psoas major (PM), and Semimembranosus (SM) in plant A 76

Table 3: Effects of carcass size, chilling rate, and electrical stimulation on pH decline in the Longissimus lumborum (LL) in plant A.. 77

Table 4: Effects of carcass size, chilling rate, and electrical stimulation on pH decline in the Psoas major (PM) and Semimembranosus (SM) in plant A..................................... 78

Table 5: Effects of carcass size, chilling rate, and electrical stimulation on temperature in the Longissimus lumborum (LL), Psoas major (PM), and Semimembranosus (SM) in plant B.. 79

Table 6: Effects of carcass size, chilling rate, and electrical stimulation on temperature in the Longissimus lumborum (LL), Psoas major (PM), and Semimembranosus (SM) in plant B.. 80

Table 7: Parameter estimates of the exponential cooling model fitted to deep tissue temperature in plant B... 81

Table 8: Test comparing the rates constant for deep tissue of treatment groups against that of the reference group (LCCNES) in plant B 82

Table 9: Parameter estimates of the exponential cooling model fitted to surface tissue temperature in plant B 83

Table 10: Test comparing the rates constant for surface temperature of treatment groups against that of the reference group (LCCNES) in plant B 84

Table 11: Parameter estimates of the exponential cooling model fitted to deep tissue temperature in plant A 85

Table 12: Test comparing the chilling rates for deep tissue of treatment groups against that of the reference group (LCCNES) in plant A 86

Table 13: Parameter estimates of the exponential cooling model fitted to surface tissue temperature in plant B 87

Table 14: Pairwise comparisons of the rates of chilling for surface temperature of treatment groups against that of the reference group (LCCNES) in plant A........ 88

Table 15: Descriptive statistics for grading data in plant A........ 115

Table 16:Descriptive statistics for grade data in plant B 116

Table 17: Effects of carcass size (H vs L), electrical stimulation (ES vs NES), and chilling rate (CC vs DC) on sensory panel ratings in plant A 117

Table 18: Effects of carcass size (H vs L), electrical stimulation (ES vs NES), and chilling rate (CC vs DC) on sensory panel ratings in plant B 118

Table 19: Effects of carcass size (H vs L), electrical stimulation (ES vs NES), and chilling rate (CC vs DC) on WBSF and SSF values in plant A 119

Table 20: Effects of carcass size (H vs L), electrical stimulation (ES vs NES), and chilling rate (CC vs DC) on WBSF and SSF values in plant B 120

Table 21: Parameter estimates for exponential decay model fitted to least squares means of WBSF of treatment groups for plant A 121

Table 22: Parameter estimates for exponential decay model fitted to least squares means of WBSF of treatment groups for plant B 122

Table 23: Rate of change in Warner-Bratzler shear force (WBSF; kg/instantaneous unit of time) for treatment groups in plant A as estimated by nonlinear regression models1 fitted to least squares means of treatment groups 123

Table 24: Rate of change in Warner-Bratzler shear force (WBSF; kg/instantaneous unit of time) for treatment groups in plant B as estimated by nonlinear regression models1 fitted to least squares means of treatment groups 124

Table 25: Warner-Braztler shear force (WBSF) for treatment groups at 14 d, aging response (change in shear force through 35 d), percentage of that change completed at each postmortem aging period, response class, and day 95% of the aging response was completed in plant A 125

Table 26: Warner-Braztler shear force (WBSF) for treatment groups at 14 d, aging response (change in shear force through 35 d), percentage of that change completed at each postmortem aging period, response class, and day 95% of the aging response was completed in plant B 126

Table 27: Effects of carcass size, chilling rate, and electrical stimulation on temperature in the Longissimus lumborum (LL), Psoas major (PM), and Semimembranosus (SM) in plant A 150

Table 28: Effects of carcass size, chilling rate, and electrical stimulation on pH in the Longissimus lumborum (LL), Psoas major (PM), and Semimembranosus (SM) in plant A 151

Table 29: Effects of carcass size, chilling rate, and electrical stimulation on tenderness attributes (WBSF, SSF) of the Longissimus lumborum (LL) plant A 152

LIST OF FIGURES

Figure 1: Fitted curves overlaid over observed deep tissue temperature decline of treatment groups in plant B 87

Figure 2: Fitted curves overlaid over observed surface temperature decline of treatment groups in plant B 88

Figure 3: Fitted curves overlaid over observed deep tissue temperature decline of treatment groups in plant A 89

Figure 4: Fitted curves overlaid over observed temperature decline of treatment groups in plant B 90

Figure 5: Predicted Aging curve vs actual WBSF in plant A 123

Figure 6: Predicted aging curve vs actual WBSF in plant B 124

INTRODUCTION

Increased carcass sizes and weight are contributing to challenges facing beef packers as heavier carcasses are hard and take substantially more time to appropriately chill (Agbeniga and Webb, 2018). The last 5 National Beef Quality Audits (NBQA) tracked hot carcass weight (HCW) over the last 25 years and showed that the beef industry has been putting on average 1.81 kg on each carcass every year (Boykin et al., 2017a). This corresponds to a 13 % increase in carcass weight between 1991 and 2016. In 2000, the frequency of carcasses with a HCW exceeding 431 kg (considered heavy weight) was 4.6% (McKenna et al., 2002). By 2016, that proportion increased to 25.7% (Boykin et al., 2017a). Furthermore, increases in carcass weight, which have been associated with an increase in ribeye area (Boykin et al., 2017a) and carcass length (Klauer et al., 2018) can contribute to alter chilling rates (Agbeniga and Webb, 2018).

The increased carcass size and weight has not always been followed by improvements in facility capabilities and management practices to account for the extra weight and size. For instance, most of existing blast chillers have primarily been built to hold and cool down smaller and lighter carcasses (Maddock, 2015). Thus, it could be challenging to achieve similar rates of chilling in heavy and light carcasses under identical conditions. More specifically, differences in carcass weight and size could influence the rate of deep tissue (center of the round or chuck) temperature decline (Aalhus et al., 2001, Kuffi et al., 2016). A consequence of an inadequate chilling rate or at least variations in the rate of chilling is the development of an aberrant meat quality (Bendall, 1973).

With the understanding that carcass size has increased significantly during the last several decades and could continue to grow as a result of improvements in genetics, nutrition, reproductive

technologies, health management, and other technologies (Dahlen et al., 2014, Maddock, 2015, Maples et al., 2017, Maples et al., 2018), the industry must continue to the investigate the effects of chilling rates and post-harvest processes (i.e. electrical stimulation) on tenderness, temperature decline, pH decline, purge loss (juiciness), and color.

Smith et al. (2008) summarized post-harvest practices for enhancing beef tenderness and clearly documented that multiple approaches and combinations of chilling rates and electrical stimulation can be utilized to achieve improved tenderness in beef muscles. Interestingly, this review highlighted that both slow and rapid chilling rates can be manipulated to improve beef tenderness. The authors reported improvements in tenderness of 10 to 40 % depending on the muscle type because of slow chilling rates. These improvements were attributed to the prevention effects of slow chilling rates on cold-shortening (Smith et al., 2008b). Furthermore, these authors suggested that very fast chilling could produce tender meat because of a combination of proteolysis and crust freezing. Similarly, both high and low-voltage electrical stimulation, when combined with appropriate chilling rates, were shown to improve tenderness and muscle color (Smith et al., 2008). The resulting physical disruption, accelerated rate of postmortem pH decline, and hastening of rigor mortis and proteolysis of high voltage electrical stimulation cause the muscle to be more tender. Low voltage has been shown to facilitate the tenderization process through prevention of muscle shortening during the development of rigor mortis (Smith et al., 2008b). However, few studies have examined the impacts of the current variability in carcass size and weight on beef quality in response to postmortem practices. Therefore, this study aims to identify the relationship between carcass size, chilling rate, and electrical stimulation to ultimately make recommendations, which can help to appropriately manage the postmortem processing of today's carcasses.

CHAPTER I

REVIEW OF LITERATURE

Electrical stimulation

Electrical stimulation (ES) has been an industry-wide adopted technology that is credited with significant improvements in quality of beef and sheep meats. Electrical stimulation consists in passing an electrical current through the carcass. Electrical systems deliver impulses that mimic nervous potential action and cause the muscles to enter a cycle of contraction and relaxation. The resulting acceleration of postmortem metabolic processes, and the physical disruption of the muscle tissues have been suggested to cause significant improvements in meat quality. Studies indicated that various degrees of objective (shear force values) and subjective (sensory panel) tenderness improvements had been achieved using different ES inputs and depending on carcass characteristics (Stiffler et al., 1982). Meat quality improvements of electrical stimulated red meat species include but are not limited to tenderness, grades, color and color stability, and water holding capacity.

It has been suggested that Franklin Roosevelt was the first to have noticed that electrocuted turkey appeared to be more tender than non-stimulated ones (Devine et al., 2014). Early ES commercial applications mushroomed in the lamb industry where ES prevented cold induced shortening and consequent toughening. Australian and New-Zealand producers used to freeze sheep and lamb to ship it overseas. They noticed that the meat significantly shortens and toughens at thawing. When ES was applied to the carcasses before to freezing, the issue was significantly overcome and tenderness variability reduced (Thompson et al., 2005, Tooley et al, 2006). As ES

makes its way into the sheep and lamb industry, several research studies on beef suggested that variability in beef tenderness could also be limited by using ES. In the US, beef plants adopted and introduced electrical systems in their processes following recommendations by Texas A&M researchers (McKeith et al., 1981, Savell et al., 1978a, Savell et al., 1978b, Savell et al., 1979, Savell et al., 1977). Their work had shed light on tenderness and quality improvements of stimulated carcasses over non-stimulated counterparts, advantages that could profit the industry. Furthermore, they showed that stimulated carcasses appeared to be similar to "over the weekend" carcasses that grade a significantly higher proportion of choice than carcasses harvested during the week (Smith et al., 2005, Strydom et al., 2005, Ferguson et al, 2000). Roeber et al. (2000) showed that electrical stimulation was more beneficial to middle cut muscles than muscles of the round. As a result of the study, electrical systems in the USA are purposely designed to deliver electrical output to the muscles of the middle cut.

Effect of electrical stimulation on postmortem metabolism

An important body of literature indicates that ES primarily exerts its effect by accelerating postmortem glycolysis and pH decline and hastening rigor mortis. In addition, ES contributes to meat tenderization by early activation of proteolytic enzymes, in particular the calpain and cathepsin systems (Hwang et al., 2003).

In a living animal, muscle tissues are supplied with oxygen and the aerobic oxidation of substrates generates sufficient energy to sustain muscle function and maintain homeostasis. Energy substrates such as glucose, glycogen, fatty acids, and amino acids are broken down to several intermediary compounds in the cytoplasm. The end product of glucose degradation or glycolysis in the cytoplasm of muscle cells is pyruvate. Glycolysis also generates reducing agents such as NADH and FADH2. Pyruvate and other intermediary products are transported into the

mitochondria where they are further processed in the tricarboxylic acid cycle (TCA). In the presence of oxygen, the reducing power is oxidized in the electron transport chain and yields water and carbon dioxide. The process is exergonic and the released energy is made available for cell function as adenosine triphosphate (ATP). The whole process is known as aerobic metabolism (Ferguson and Gerrard, 2014, Scrutton and Utter, 1968).

When the animal is bled, its muscle cells lose their ability to maintain homeostasis. Oxygen supply to the muscles is interrupted and removal of metabolic waste is rendered unavailable. To reinstate homeostasis and provide some energy to essential functions, the muscles will switch from aerobic to anaerobic metabolism. More specifically, anaerobic glycolysis proceeds and glucose and glycogen are converted to lactate, protons (H+), and ATP. The energy output generated under such conditions is relatively low compared to that of aerobic metabolism. As lactate and hydrogen protons accumulate in cell's cytosol, the muscle pH drops from the homeostatic level 7.0 - 7.2 to 5.4 – 5.8 (Honikel, 2014, Bendall, 1973). When energy is available, the muscle contracts and relaxes and remains extensible. Depletion of energy stores leads to rigor mortis or stiffness of death characterized by a gradual, then complete and permanent loss of muscle extensibility.

The decline in pH and the onset of rigor mortis are greatly influenced by environmental conditions namely temperature (Bendall, 1973). Under commercial processing conditions, the temperature of carcasses is reduced to levels where microbial growth is less likely to occur and present a safety issue. The interaction between temperature and pH decline is critical to the rate of glycogen depletion and the onset of rigor mortis. A very fast and low temperature decline slows down anaerobic glycolysis and pH drop and may prevent rigor onset. This occurs when muscle meats are frozen before to rigor onset. Rigor is resumed at thawing and is accompanied by an extensive shortening and purge loss. The phenomenon is known as thaw rigor and is highly

undesirable for the meat industry (Devine et al., 1999). If the muscle temperature drops below the 12-15°C range before muscle pH reaches 6, cold induced shorting occurs. The degree of shortening associated with shortening is lower than that produced by thaw rigor. When pH decline is too slow, rigor onset is hastened and occurs at high pH (above 6) and high temperatures (above 35°C). The corresponding shortening, termed heat induced shortening, is lower compared to that of cold induced shorting (Devine et al., 1999, Wheeler and Koohmaraie, 1994).

Applying electrical stimulation to carcasses has the primary consequence of inducing a series of muscle contraction and relaxation that exhausts very rapidly available ATP and energy substrates such as glucose and glycogen. Previous reports of the effect of ES on pH decline indicate that the drop in pH as well as the rate of pH decline are quickened by ES (Chrystall and Devine, 1978, Chrystall and Devine, 1983, Chrystall et al., 1980, Chrystall and Hagyard, 1976, Devine et al., 2014, Savell et al., 1977, Smith, 1985). The electric current provides sufficient energy to overcome the energy of activation required for the muscle to be excited and enter contraction and relaxation cycles.

Energy activation in non-stimulated carcasses has been estimated under various conditions and had been found to vary between 40 to 110 kJ mol^{-1}, meaning that as much energy is necessary for the activation of enzymes (myosin ATPase) involved in muscle contraction (Devine et al., 2014). Under normal conditions, this may take long time to occur. In stimulated muscle, activation energy has been estimated to be lower than 50 kj.mol^{-1}, suggesting that enzyme activity is greatly induced by ES (Devine et al., 2014). This may explain the changes in the initial rate of pH fall and the rate of pH decline thereafter between non-stimulated and stimulated muscles. Daly (1997) reported that stimulated muscles showed a 50 to 75% faster pH fall than their non-stimulated counterparts. The initial sharp drop in pH, estimated to be around 0.4 to 0.5 pH units, has been reported to occur

100 to 150 times faster than the rate of normal pH decline (Chrystall and Devine, 1983). It has been suggested that this initial faster rate decreases significantly after a certain time post stimulation period but remains 1.5 – 2 times faster than the rate observed in non-stimulated carcasses. Kondos and Taylor (1987) reported a 32 % decrease in ATP concentration in stimulated (low voltage 32 V, for 90 sec) carcass sides compared to non-stimulated sides. Electrical stimulation was applied within 30 min postmortem. These authors also reported an initial 18% pH fall in stimulated sides as compared to non-stimulated counterparts. They further observed that ES increased the rate of glycogen depletion.

The extent of the initial pH fall, and the following increased rate of pH decline were found to be responsible for hastening rigor mortis (Hwang et al., 2003, Devine and Chrystall, 1984, Newbold and Small, 1985). In parallel to ATP depletion, Kondos and Taylor (1987) found lactate concentrations to increase in reverse fashion of the disappearance of glycogen concentrations. These observations were made on samples excised from the *M. sternocephalicus* (Kondos and Taylor, 1987). Similarly, Kim et al. (2013) found a more rapid pH decline in *Longissimus dorsi* (LD) muscle of carcass sides stimulated (100V, 60-Hz, 30s) within 90 min of exsanguination. However, they reported that ES did not affect pH decline in the superficial portion of *Semimembranosus* (SSM). The authors stipulated that different muscles may respond differently to electrical stimulation.

The mechanisms whereby ES induces faster pH decline remain unclear. It has been suggested that the acceleration of pH fall may be due to an increase in phosphorylase (a) activity (Horgan and Kuypers, 1985). Other authors postulated that an increased afflux of calcium released from the sarcoplasmic reticulum might explain the rapid drop in pH in stimulated muscles (Daly, 1997).

The rate and extent of pH decline are dependent upon the muscle and the parameters of the ES applied. Several studies previously reported that voltage, frequency, wave, and current form, and the time of application and duration of stimulation are primary determinants of the effects of ES on muscle metabolism and structural changes. More specifically, these factors greatly influence the rate and extent of pH decline in stimulated muscles and affect meat quality (Chrystall and Devine, 1978, Chrystall and Devine, 1983, Chrystall and Devine, 1985, Chrystall and Hagyard, 1976, Davey et al., 1976b, Devine and Chrystall, 1984, Savell et al., 1977). Bouton et al., (1980) reported that high voltage ES had a more significant influence on pH fall than medium, low, or ultra-low voltage stimulation. The acceleration of postmortem metabolism by ES has been suggested to be the main underlying mechanism of meat quality improvements.

The ES and prevention of cold shortening

The development of ES in the lamb industry in New Zealand and Australia in the early 1970s was based on the potential of the technology to preclude thaw rigor and cold shortening, and help manage tenderness variability (Simmons et al., 2008, Chrystall and Devine, 1985, Chrystall et al., 1980). Cold shortening as a result of rapid chilling is consequential to severe muscle contraction at the onset of rigor mortis. Rapid chilling conditions that enable muscle temperature to drop below 15°C while muscle pH remains above six cause cold induced shortening and toughening (Simmons et al., 2008). Under rapid chilling conditions, energy depletion (ATP) and anaerobic glycolysis are seriously reduced, which typically leads to low temperature/high pH conditions conducive to cold shortening. The passing of an electric current through the carcasses induces cycles of muscle contraction and relaxation that rapidly exhaust ATP stores and accelerate glycolysis. Thus, electrical stimulation has been used in the meat industry to hasten postmortem metabolism and

enable a rapid pH decline. This allows the meat industry to chill carcasses rapidly while avoiding the risk of excessive muscle contraction at the onset of rigor mortis (Davey et al., 1976b).

Additionally, the economic significance of cold induced shortening/toughening in relation to tenderness is only relevant when the muscle shortens more than 20 % of its initial length (Davey et al., 1967). This requires significant levels of ATP and glycogen at the onset of rigor mortis, conditions that are not likely to exist in electrically stimulated carcasses. In a similar way, thaw rigor shortening is precluded in frozen pre-rigor cuts. The depletion of glycogen and ATP by ES before to muscle freezing inhibits the typical excessive muscle contraction and accompanying shortening that occur at thawing. Also, muscle fibers subjected to the extensive muscle contraction elicited by ES remain extended, which may prevent to a certain degree muscle shortening (Simmons et al., 2008, Thompson et al., 2006).

The ability of electrical stimulation to prevent cold shortening in beef has also been questioned by some researchers as current commercial conditions are less likely to be conducive to cold shortening (Bekhit et al., 2014, Simmons et al., 2008). Under current commercial chilling conditions, most beef carcasses tend to reach the ideal temperature/pH window of 10 to 15°C when pH is still above 6.

Effects of electrical stimulation on structural changes

It has primarily been suggested that tenderness improvements resulting from ES application were due to damaging effects of ES on myofibrillar structure (Pearson and Dutson, 1985, Savell et al., 1978a, Will et al., 1980, Takahashi et al., 1987). Savell et al. (1978) and Luo et al. (2008) reported stretched areas and physical sarcomere disruptions associated with contracture bands. Microscopic observations revealed disrupted patterns and ill-defined I-bands and Z-lines that may be comparable to microscopic observations of aged muscle (Takahashi et al., 1987, Will et al.,

1980, Savell et al., 1978a). The damage caused to the muscle depends on the electrical stimulation parameters.

The consensus among meat scientists is that high contraction forces induced by ES are responsible for muscle structural damage (Simmons et al., 2008). However, consensus is yet to be achieved when it comes to whether the physical muscle tear/disruption is sufficient to explain tenderness improvements observed in stimulated muscles. Indeed, many studies suggest that structural damage may not be sufficient to explain tenderness improvements. Moreover, several authors proposed that the mechanism of ES enhanced tenderization of muscle meats may be due to the ability ES to activate proteolytic enzymes early postmortem and hasten proteolysis, when temperature and pH are more favorable (Hwang et al., 2003). Hence, the physical disruption may lead to an acceleration of enzymatic proteolysis more so than directly affect tenderness. The disruption of muscle cells may enhance contact between proteolytic enzymes and their substrates, in particular lysosomal enzymes which are released into the cytosol (Devine et al., 2014).

Effects of electrical stimulation on postmortem proteolytic activity

It is well accepted that the merits of electrical stimulation also include the improved rate of muscle tenderization and rapid resolution of rigor mortis. The view that ES improves meat tenderness and tenderization via an acceleration of postmortem proteolysis is based upon the fact that conditions created by ES application promote the massive release of calcium ions (Ca++) from the sarcoplasmic reticulum. Under normal conditions, muscle contraction occurs in response to a release of calcium ions from the sarcoplasmic reticulum and activation of myosin-ATPase. The extra free calcium is sequestered back into the sarcoplasmic reticulum. This is possible because the calcium ion pump functions normally. In stimulated postmortem muscle, the calcium ions released during muscle contraction and/or by disrupted sarcoplasmic reticulum membrane will

have a direct impact on the activation the calpain system under high pH (> 6.5) and temperature (≥ 30°C) conditions (Devine et al., 2014, Adeyemi and Sazili, 2014). It has been shown that ES and the high pH/temperature conditions lead to irreversible calcium pump damage (Devine et al., 2014). Hence, the calcium released in the cytosol could no longer leave it but will rather be used to activate and sustain proteolysis.

Evidence indicates that free calcium concentrations could increase by as much as 100 $\mu mol\ l^{-1}$ following ES, suggesting that higher levels of calcium are available for muscle contraction and early proteolysis (Devine et al., 2014). The release of calcium lowers the calcium ion requirement for calpains, which triggers their early activation and leads to early postmortem proteolysis. Under high pH and temperature, calpain activity is favored.

The early tenderization of stimulated muscle could also be explained by the fact that proteolysis admittedly starts at rigor onset. Since ES accelerates pH decline and hastens rigor onset, aging starts earlier as a result. One important feature of rapid pH decline is that it accelerates autolysis and activation of μ - calpains. This results in accelerated proteolysis of calpain substrates (Huff Lonergan et al., 2010).

The disruption of muscle structure as a result of electrical stimulation could improve tenderness and accelerate the tenderization process by releasing cathepsins whose proteolytic activity postmortem has been acknowledged. However, it is unclear whether the activity of lysosomal proteases contributes significantly to tenderness improvements in stimulated muscles.

The efficacy of ES depends on many factors including the type of ES (voltage, frequency, duration of the pulses, shape, etc), time of application, chilling regime, animal (age, sex, weight), pre-slaughter handling, ultimate pH, stress, feeding, temperature of the muscle, muscle fiber type, initial glycogen stores, etc.

Differential response of muscles to ES

Muscle profile varies greatly across the carcass based on anatomical, physiological, biochemical location, and functional features (Pette and Staron, 1990). Therefore, it makes sense that muscle response to postmortem management practices such as ES differs between and within muscles. The composition of skeletal muscle consists of red, white, and intermediary muscle fibers (Kirchofer et al., 2002, Ryu and Kim, 2005). The proportion of each muscle fiber type in the muscle is variable. Each fiber type exhibits a different metabolic pathway, which substantiates the complexity and variability in the muscle response to postmortem quality interventions (Listrat et al., 2016, Stolowski et al., 2006). More specifically, postmortem glycolysis and rigor onset vary significantly in white, red, or intermediary muscle fibers. Hence, postmortem interventions that affect postmortem metabolic processes may yield different outcomes depending on the relative composition of the muscle. This may explain the conflicting reports on the effect of ES on different muscles.

It has been reported that white muscle fibers are less subject to cold shortening than red muscle fibers (Devine et al., 1984, Lawrie, 2006a). Cold-induced shortening susceptibility of red muscle fibers has been suggested to be caused by the inefficiency of red muscle fibers to resequester calcium (McKee, 2004, Smulders et al., 1990). In consequence, the routes of ES induced tenderness improvements in white muscle fibers may not involve prevention of cold shortening. However, the high glycolytic potential of white muscle fibers make them more susceptible to the impact of ES than red muscle fibers. Devine et al. (1984) showed that ES had a more profound effect on white and intermediary muscle fibers than red ones. Similarly, Kim et al. (2013) found that LD was more affected by ES than SM.

Effects of electrical stimulation on meat color

Meat color is singlehandedly the most important driver of meat choosing at the point of sale. Consumers of meat base their purchasing decision on the bright cherry red color of beef assuming that it indicates freshness and wholesomeness. A deviation from the cherry red color is synonymous of spoilage or a quality alteration, leading ultimately to the product being discriminated against. For example, meat discoloration has been associated with an annual $1 billion revenue loss (Smith et al., 2000). Hence, strategies to improve meat quality should include careful management of their impacts on meat color. Electrical stimulation has been primarily used in the industry as a means to improve tenderness and counteract the detrimental effect of cold - induced shortening/toughening (Chrystall and Devine, 1985). Yet, an early work by Smith (1985) showed an improvement in grade factors namely the color of the LD at grading. Several studies thereafter have corroborated and reported different levels of ES-mediated color enhancements across different livestock species including but not limited beef (Smith, 1985, McKeith et al., 1981, McKenna et al., 2007), lamb (Polidori et al., 1999), and pork (Taylor and Martoccia, 1995).

The mechanism whereby ES improves color is unclear. However, it has been suggested that ES induced color improvements result from the faster rate and extent of pH fall. The rapid pH decline while muscle temperature remains still high enough leads to a slight protein denaturation that could alter light absorption and reflectance properties of the meat. As a result, the meat appears lighter in color (Huff-Lonergan and Lonergan, 2005). Some studies have found no differences in color stability between stimulated and non-stimulated groups (Ledward et al., 1986, Mombeni et al., 2013) while some others have shown that ES reduced color stability (Wiklund et al., 2001). While the mechanisms remain unclear, it has been suggested that the damaging effect of ES on

enzymatic systems and sarcoplasmic protein denaturation may be implicated (Renerre and Bonhomme, 1991).

Types of electrical stimulation

Since its landslide application, various ES types have been developed and could fit under high, medium, or low voltage categories. The basis of this distinction resides in the electrical output delivered to the carcass. Typically, low voltage ES (LVES) is carried out at voltages no greater than 100 V, while high voltage ES (HVES) requires voltages greater than 110 V (Adeyemi and Sazili, 2014). A plethoric number of parameters are reported in the literature including amperage, frequencies, pulse width, duration, time of application postmortem, electrode type and position, etc. Furthermore, ES units/systems can also be classified as batch or continuous, manual or automatic. Batch systems typically utilize electrode probes that are attached to carcass extremities, one to the hind region and the other to the head or neck (Devine et al., 2014, Stiffler et al., 1983). Automated batch systems requiring no human manipulation consist of electrode bars that can move out to make contact with the carcass. Such systems utilize shielded cabinets carcasses pass through to be stimulated (Devine et al., 2014). Continuous ES apparatuses are composed of a frame that extends from an inlet to an outlet and where stationary rubbing electrodes or moving series of electrodes apply a current to the carcass. The current flow runs from an upper ground to lower ground in contact with carcasses.

In the USA, Colorado State University and Texas Tech University researchers developed ES apparatus that delivers electrical output to the middle region of beef carcasses (Roeber et al., 2000, Allen et al., 2001). The work of Roeber et al. (2000) was instrumental to the development of ES systems that target the muscles of the middle region of the carcass and are now utilized in almost all plants in the USA.

Regardless of the ES system being utilized various factors must be considered to achieve high quality meat. Carcass size, carcass fatness, contact surface (whole, sides, or muscles) determine the efficacy of the electrical treatment on the final product quality (Devine et al., 2014, Adeyemi and Sazili, 2014, Roeber et al., 2000).

Postmortem glycolysis

The skeletal muscle of mammals plays an essential role in the living animal. It provides support, composure, maintenance, heat during inclement weather, and more substantially mechanical energy. Skeletal muscle is also a source of high quality nutrients for humans. It has played an important role in human brain evolution as suggested by anthropological evidence (Pereira and Vicente, 2013, Mann, 2007). Animal proteins provide to the human body all the amino acids required for its growth, development, and maintenance in sufficient quantity and quality (Aberle, 2001). Consumption of muscle meats should remain an important part of a balanced diet despite recent concerns.

Epidemiological data seem to indicate some association between the consumption of red meat and an increased risk of certain cancer types, cardiovascular, and metabolic disorders (Pereira and Vicente, 2013). However, muscle meats remain an unequivocal source of such micronutrients as iron, selenium, zinc, phosphorus, and the B-complex vitamins. The proximate composition of muscle meats is approximately 70 % water, 20% proteins, 2 to 5% fat, 1 % carbohydrates, and less than 1% vitamins and minerals (ash).

The conversion of muscle to meat is a complex process that involves biochemical, physiological, and physical changes. The understanding of these postmortem bioprocesses is key to ensuring production of high quality and safe meat. The natural biological variability that exists within and between muscles in an animal and across animal species substantiates anthropic

interventions to produce high-quality meat. This is the foundation of meat science and the development of many technologies, processes, and managements tools used to improve meat quality. More specifically, the control of postmortem metabolism is one of the most determining factors in the successful conversion of muscle to meat.

Biochemical changes during postmortem metabolism

Biochemistry of a living muscle

Living skeletal muscle is a highly specialized tissue that executes different functions in a living animal. It is important to understand muscle structure and the complex biochemical and physiological features that govern its activities in vivo in order to understand its implications for postmortem muscle and its conversion to meat.

Muscle structure

The skeletal muscle of animals is composed of muscle cells or myofibers that are highly organized in a hierarchal scheme. Each muscle fiber is made up of structural contractile units or sarcomeres responsible for the striated appearance of mature myotubes. Myofibrils are covered by a layer of a connective tissue membrane called endomysium. Contractile proteins myosin and actin and associated proteins constitute the makeup of sarcomeres and are responsible for the conversion of ATP into mechanical energy. Myofibrils are grouped into muscle bundles and are encased in an epimysial sheath of connective tissue. Perimysium encloses groups of muscle bundles that form the muscle architecture. A membrane-bound structure of highly specialized subcellular organelles, the sarcoplasmic reticulum is interwoven through the myofibers. The sarcoplasmic reticulum regulates muscle contraction via regulation of calcium ions concentration (McNally et al., 2006).

The striated appearance of sarcomeres is due to an alternating alignment of light and dark bands observable under light microscopy. The light bands are called I bands and the dark ones A

bands. Each sarcomere is delimited by Z discs or Z-lines that anchor thin filaments. The light bands represent non-overlapping thin filaments. The major protein component of thin filaments is actin. The dark band represents myosin thick filaments that occupy the center of the sarcomere and can slide pass the thin filaments during muscle contraction. The center of the A band is occupied by H-band representing a lighter area where thick filaments do not overlap with thin filaments (McNally et al., 2006).

Myosin, a filamentous hexamer with two heavy chains (220 kDa) and four light chains (20 – 25 KDa), has a globular head and a rod or tail region (McNally et al., 2006). Myosin head domains contain amino acid sequences that have ATP hydrolysis and actin binding properties. Myosin rods are entangled in a coil and rope-like configuration and form the backbone of the thick filaments. The head region is projected outward perpendicular to the tail backbone of the filament.

Actin is the major component of the thin filaments and is made up of globular G-actin monomers assembled in double helical filamentous backbone (F-actin). The double strand actin backbone is characterized by ionic strength and myosin binding sites regularly distributed along its length. Regulatory proteins known as troponins T, C, and I run along the thin filaments together with is a linear-shaped protein called tropomyosin. Tropomyosin interacts with troponin C to cover or uncover myosin binding sites on actin during contraction and relaxation. The interaction between troponin C and tropomyosin is turned on and off by direct binding and release of calcium ions to troponin C.

In addition to myofibrillar (actin and myosin) and regulatory (troponins T. C, I) proteins, other constituents of sarcomeres include sarcoplasmic and structural proteins, specific innervations, and the necessary machinery to generate energy or synthesize proteins. Titin, nebulin, and desmin are three of major cytoskeletal proteins responsible for maintaining the organized architecture of

sarcomeres. Sarcoplasmic proteins include myoglobin and creatine kinase and are localized in the cytoplasm where mitochondria are also found. Mitochondria are the energy powerhouse of muscle cells. Myoglobin stores and transport oxygen within muscle cells and is responsible for meat color in a well bled animal.

It is important to notice that muscle fibers vary in diameter, vascular and neural innervation, metabolic pathway, mitochondria and myoglobin contents, glycogen concentration, and activity. Fast twitch activity, glycolytic metabolism, high glycogen concentration, and low mitochondrial and myoglobin concentrations and blood supply characterize type IIB, white muscle fibers. Type IIB muscle fibers have high ATPase activity and fatigue rapidly. Type IIA muscle fibers are intermediary and characterized by a moderate oxidative and glycolytic metabolism, myoglobin and mitochondrial concentrations, blood supply and lipid content, and a relatively moderate twitch activity. Type I muscle fibers are slow twitch, oxidative, resistant to fatigue and rich in myoglobin and mitochondria. Type I fibers have low creatine phosphate and glycogen contents.

The structural conformation of sarcomeres is the basis for the now widely accepted sliding filament theory of muscle contraction.

Muscle contraction

The sliding filament theory is based on the structural stability of the sarcomeres. In response to stimulation by a nervous potential action (PA), the sarcoplasmic reticulum releases calcium that flows into the cytoplasm. Calcium concentrations increase in the cytoplasmic milieu leading to more and more calcium ions binding with troponin C. Following binding of calcium to troponin C, troponin interacts with tropomyosin to induce a conformation change that ultimately leads to a shift in tropomyosin. As tropomyosin shifts, myosin binding sites on actin are exposed. This allows myosin heads to bind to myosin binding sites on the thin filament creating an initial

conformation change. The actomyosin cross-bridge undergoes a power "stroke" that results in actin filaments being pulled (sliding) and held onto tightly by myosin heads. As the thin filaments slide towards the center of the sarcomere, the length of the sarcomere shortens. The complex dissociates when ATP binds to myosin ATPase sites. The hydrolysis of ATP by myosin signals a reset of both thin and thick filament. The muscle relaxes. Calcium is sequestered back into the sarcoplasmic reticulum as the muscle relaxes (DiCapua, 2014, Bespalova and Tolpygo, 1991, Geeves and Holmes, 1999).

In postmortem muscle, the depletion of ATP and damage of calcium pumps prevents muscle relaxation. As a result, the muscle undergoes biochemical and structural changes that are responsible for the conversion of muscle to meat.

Postmortem glycolysis

The processes that turn animal muscle into meat start at exsanguination. At exsanguination, the removal of blood has the immediate consequence of depriving the animal muscles of the supply of energy substrates (glucose, fatty acids, amino acids) and oxygen they need, and a vehicle to remove metabolic waste. Under these ischemic and hypoxic conditions, the muscle will fight to reinstate homeostasis and maintain activity of key processes that require energy production (ATP) such us the ion pumps. Readily available substrates such as creatine phosphate and ATP present within the muscle at the time of death will be primarily utilized while the muscle switches off anabolic bioprocesses to rely only on catabolic pathways to regenerate energy (ATP). The rate of postmortem energy replenishment decreases till it stops when substrates are completely depleted and/or when environmental conditions inhibit enzymatic activity (England et al., 2013). Specifically, energy dense phosphate compounds such ATP and phosphocreatine and energy

substrates such as glucose, and glycogen are progressively used up (Honikel, 2014, Huff Lonergan et al., 2010, Matarneh et al., 2017).

The primary readily available source of energy is the creatine phosphate (PCr) pathway. Creatine phosphate is part of the phosphagen system that rapidly restores and buffers ATP levels during contraction. Early postmortem, the phosphagen system generates ATP by transferring inorganic phosphate from creatine phosphate to ADP and releasing creatine. Cytosolic phosphocreatine kinase (PCK) is responsible for catalyzing the transfer. Creatine is then rephosphorylated by mitochondrial PCK to PCr. Myofibrillar creatine phosphate kinase (CPK) converts ADP to ATP by utilizing the phosphate group on creatine phosphate. Creatine is then rephosphorylated to PCr by mitochondrial CPK under resting conditions where energy demand is low. In postmortem muscle, limited Pcr reserves are rapidly exhausted to regenerate ATP. The rate of ATP production is considerably slow, which results in accumulation of ADP. Hence, adenylate kinase (AK) is activated to continue to supply ATP by converting two molecules of ADP to ATP and AMP. AMP is deaminated to inosine monophosphate (IMP) by AMP deaminase. IMP is a dead end from the energy standpoint (Matarneh et al., 2017, England et al., 2015). It is worth noticing that the regeneration of ATP via the phosphagen system does not produce hydrogen ions (Ferguson and Gerrard, 2014, Matarneh et al., 2017). As the phosphagen system rapidly exhausts, the muscle switches from aerobic metabolism to anaerobic metabolism to provide energy. Glycogenolysis and anaerobic glycolysis become the main route of ATP production.

Glycogen is a branched polymer of glucose residues linked by α- 1, 4 and $\alpha - 1, 6$ – glycosidic bonds. The $\alpha - 1, 6$ – glycosidic branches are distributed every 8 to 12 residues. Although glycogen represents less than 1% of the muscle, its significance to the development of meat quality is substantial. Glycogen is primarily degraded to glucose by glycogen phosphorylase (GP) which

cleaves α- 1, 4 – glycosidic bonds and glycogen debranching enzyme (GDE) which hydrolyzes α – 1, 6 linkages (Matarneh et al., 2017). Under aerobic conditions, glucose is then hydrolyzed to pyruvate. Pyruvate is transferred into mitochondria where it is processed in the tricarboxylic acid cycle (TCA) and electron transfer chain to generate energy. In the postmortem muscle, the anaerobic degradation of glucose produces ATP (in lesser quantity), lactate, and hydrogen protons. Lactate is derived from pyruvate by the enzyme lactate dehydrogenase. The reaction is coupled with NAD+ regeneration, which is a step crucial for glycolysis to proceed. The accumulation of protons within the muscle cells results in pH decline and is driven by ATP hydrolysis (Matarneh et al., 2017). Due to limited substrate availability (glycogen) and the increase in undesirable conditions for glycolytic enzymes caused by the accumulation of lactate and protons, glycolysis stops at some point. The decline in pH is paralleled by the drop in ATP levels. Since biochemical reaction rates are greatly affected by temperature, chilling conditions will greatly affect the rate of postmortem glycolysis. A faster rate of chilling will slow down metabolic processes while a slower rate of chilling will hasten metabolic reactions in the muscle. Similarly, electrical stimulation hastens glycolysis and the onset of rigor mortis.

The buildup of lactate and protons is concomitantly accompanied by a steady drop in pH. Due to limited available substrates (glucose and glycogen), the developing undesirable conditions (low pH), and the buffering capacity pH decline will stop, increase slightly and plateau for the remaining of the postmortem period. It has been suggested that the unfavorable conditions slow down and eventually impair glycolytic enzymes such that their activity is stopped, hence the presence of residual glycogen in meat after it reaches its ultimate pH (Matarneh et al., 2017). The depletion of energy substrates and the plateauing of glycolysis is paralleled by physical changes that characterize meat in texture, color, appearance, flavor, tenderness, and juiciness.

Physical changes

Metabolic processes in the postmortem muscle are accompanied by physical alterations. The progressive loss of muscle extensibility and accompanying muscle shortening, as a result of rigor mortis, represent the major postmortem physical changes that have important implications for meat quality. The phenomenon is known as rigor mortis or stiffness of death. At rigor completion, the muscle is at its highest toughness. The degree of rigor shortening has been recognized as a critical indicator of tenderness. Given the importance of tenderness for consumers of red meat, the control and manipulation of rigor mortis become important management tools for meat tenderness (Hwang et al., 2003).

Rigor mortis is characterized by three phases: delay phase, rigor onset, and rigor completion. During the first hours following death (12 to 24 h in beef), the muscle remains relatively extensible due to the presence of ATP. In the presence of ATP, myosin and actin can bind and relax. This period constitutes the delay phase. As time postmortem progresses, individual muscle fibers enter rigor mortis and progressively lose extensibility as a result of a steady ATP and glycogen stores depletion. This constitutes the onset of rigor mortis. When most of the muscle fibers within a muscle enter rigor mortis, the muscle becomes completely inextensible, and rigor mortis is said to be complete. The complete loss of extensibility coincides with ATP exhaustion. In the absence of ATP, the muscle shortens and increases isometric tension (Matarneh et al., 2017, Devine et al., 1999). Myosin and actin filaments form irreversible cross-bridges. Rigor mortis can be assessed by loading and unloading a muscle and measuring its extensibility (Warriss et al., 2003, Devine et al., 1999).

The degree of muscle shortening depends on many intrinsic (glycogen level, muscle composition, and so on) and extrinsic factors such as fiber type, muscle location, chilling rate, and electrical stimulation.

Resolution of rigor mortis

At the completion of rigor mortis, the muscle is shortened, and the meat is at its highest degree of toughness (Devine et al., 1999). The resolution of rigor mortis is not only important for tenderness improvement but also for flavor development. This process is referred to as aging or conditioning and has been extensively studied (Koohmaraie et al., 1988a, Koohmaraie et al., 1991, Huff-Lonergan et al., 1996, Lametsch et al., 2002, Ilian et al., 2004, Morgan et al., 1993) and reviewed elsewhere (Huff Lonergan et al., 2010, Ouali, 1990). During rigor resolution or attenuation, the muscle myofibrillar matrix is subjected to some degradation that weakens its structure.

It is accepted that enzymatic proteolysis is the major contributor to rigor mortis resolution and improvements in tenderness during postmortem aging (Huff Lonergan et al., 2010). Many enzymatic systems in meat have been investigated as possible effectors of meat tenderization. However, for enzymes to be recognized as involved in the postmortem proteolysis, they must be endogenous to muscle, mimic *in vivo* degradation patterns in *in vitro* models , and have access to muscle substrates according to the criteria developed by Koohmaraie (Koohmaraie and Geesink, 2006). The main proteolytic enzymes fulfilling these criteria and determined to be active in postmortem muscle are: the calpain system, caspases, cathepsins, and proteasomes.

The calpain system is recognized as the major driver of postmortem proteolysis (Koohmaraie et al., 1988a, Koohmaraie et al., 1991). In this family of cysteine proteases, the main enzymes of interest consist of μ and m calpains and their endogenous inhibitor calpastatin. Much of protein

degradation (mainly cytoskeletal proteins such as titin, nebulin, and desmin) occurring within the first 72 hours postmortem is attributed to the action of μ calpain (Koohmaraie et al., 1988a, Koohmaraie, 1992, Dransfield et al., 1992). The evidence supporting this assertion came from studies on μ calpain knockout mice (Kent et al., 2004). A relationship between the ratios of calpastatin to calpain and meat tenderness or tenderization has been found that showed the importance of the enzyme and its inhibitor on tenderness of beef. This was supported by studies with beta agonists supplemented cattle and callipyge gene lambs. These animals had higher calpastatin activity than their control counterparts and had been found to produce tougher meat.

The role of cathepsins has primarily been suggested to be more important than that of the calpain system in postmortem proteolysis. However, recent evidence seems to refute such contention based on three arguments. First, cathepsins are lysosomal and do not have access to their substrates. Secondly, their target substrates (actin and myosin) are not degraded during postmortem. Lastly, weak to no associations were found between their activity and postmortem tenderization (Koohmaraie and Geesink, 2006).

Caspases have recently gained attention as contributors to postmortem proteolysis since suggested by Ouali (2006). Caspases are the major enzymes involved programmed cell death or apoptosis. During apoptosis, the muscle cells are dismantled, which may contribute to destabilizing muscle physical structure. Evidence suggests that ischemic and hypoxic conditions in postmortem muscle are likely to induce apoptosis during the few first hours following death and contribute to meat tenderization.

The role of proteasome in postmortem tenderization has been suggested but more research is needed in this area.

Issues during postmortem metabolism

The rate and extent of postmortem metabolism, as previously stated, is greatly influenced by various environmental and intrinsic conditions. The control of postmortem glycolysis is central to producing high quality meat. Therefore, inadequate management will result in issues and quality defects that can have serious economic implications. These quality defects are well characterized in meat of different livestock species. The control and management of postmortem practices is an important part of the total quality management of beef. The economic margin for beef producers may be greatly affected by inconsistencies in the quality of the product. Hence, any factor that can cause a deviation from a perceived quality of beef is of great concern to producers. A review of different factors influencing the rate and extent of postmortem glycolysis is available in the literature (Juárez et al., 2012, Olivant, 1955, Ferguson and Gerrard, 2014). Changes in ATP, lactate, pH, and glycogen have been central to the estimation of postmortem metabolism. The pH of well bled ruminants falls from 7.2 to 5.4 -5.8 within 24 to 48 h in a curvilinear fashion. The relationship of temperature to pH decline is the main postmortem factor that can negatively affect meat quality.

Cold shortening

Biochemical reaction rates are greatly influenced by temperature. Hence, postmortem metabolic reaction rates depend on environmental factors such as temperature, air chilling velocity, and ambient humidity, and animal factors such as carcass size, subcutaneous fat thickness, and muscle location (Ferguson and Gerrard, 2014, Savell et al., 2005). Chilling carcasses has a primary purpose of removing heat and preventing bacterial growth (Savell et al., 2005). It is well documented that chilling has a tremendous influence on the development of meat quality (Savell et al., 2005, Ferguson et al., 2001). It is closely related to the degree of pH decline. To achieve

high quality in meat, heat removal of carcasses should be managed such that when the pH reaches 6, the muscle temperature is in the range of 10-15°C. Such conditions result in minimal shortening of the muscle (less than 15% of its original length), thus a more tender meat (Savell et al., 2005).

Very rapid rate of chilling resulting in temperatures at pH 6 below 10-15°C will result in cold shortening of the muscle in as much as 50% of its initial length (Locker and Hagyard, 1963). The degree of shortening increases with each drop in temperature below the 15°C cutoff. At low temperature/high pH conditions, sarcoplasmic reticulum (SR) membrane is destabilized and releases a load of calcium ions (Ca++) that triggers muscle contraction before the muscle enters rigor mortis. Also, the SR is unable to sequester back calcium ions (Ca++). The degree of rigor onset at the time of freezing is critical for the rate and degree of muscle shortening (Ertbjerg and Puolanne, 2017, Marh and Leet, 1966). This originates the contraction that results in muscle shortening and subsequent toughening (Locker and Hagyard, 1963, Wheeler and Koohmaraie, 1994). Cold shortening is more rapid early postmortem if carcasses are very rapidly or blast chilled. As discussed earlier, electrical stimulation by hastening depletion of ATP, glycolysis, and rigor onset has been utilized to prevent in cold shortening in ruminant muscles.

Thaw rigor

During thawing of frozen pre-rigor meat, the presence of ATP and glycogen causes contractions and subsequent thaw rigor shortening or toughening. The muscle shortens 60 to 80% of its original length (Locker, 1985). This is influenced by the rate of freezing. Slow freezing rate generates big ice crystals that are more detrimental to cell membranes. Disruption of cell membranes (sarcoplasmic reticulum membrane) leads to a massive release of calcium ions during thawing. Consequently, a massive contraction occurs since ATP is available. This leads to the huge shortening of the sarcomeres. This is accompanied by huge purge loss (Locker, 1985).

Heat shortening

When temperatures at pH 6 are above 35°C, heat - induced shortening or rigor shortening occurs and can account for 30% reduction in sarcomere length (Locker and Hagyard, 1963). Compared to cold shortening, fewer muscle fibers are recruited during heat induced shortening, hence the lesser degree of shortening. At high rigor temperatures, muscle fibers in the rigor mortis state exert a restraining effect on the recruitment of new muscle fibers into the rigor mortis state.

Effects of pre-harvest conditions on glycogen depletion

It has been shown that preharvest stress is the main reason for dark cutting condition as it results in low glycogen levels. Glycogen breakdown mechanisms in a living animal are under hormonal or substrate levels control. Any physical, physiological, or environmental event that alters one or the other may result in an atypical ultimate pH decline, specifically if the animal is not allowed to replenish glycogen before slaughter (Tarrant, 1989). Tarrant (1989) indicated that either adrenergic action or muscle contraction or both mechanisms drive rapid glycogen catabolism.

Glycogen metabolism postmortem generates lactate which is responsible for pH decline. High levels of glucagon maintain gluconeogenesis active in order to supply glucose - dependents tissues. Thus, depletion of glycogen stores ensues. Also, the release of epinephrine under any stress conditions has been shown to induce rapid glycogen breakdown and decrease in glycogen synthesis in muscle tissues (Dietz et al., 1980). Adrenergic hormones have been shown to induce glycogen breakdown by activating phosphorylase (a) through the adenylate cyclase – Camp mechanism (Tarrant 1989). In the long term, the animal glycogen stores are exhausted. During starvation, the animal uses up slowly his glycogen stores to supply glucose dependent tissues.

Prolonged starvation of the animal will lead to low glycogen levels prior to slaughter and subsequently to dark cutting meat.

Previous research has indicated that stress is an important factor determining glycogen levels at the time of slaughter (Grandin, 1980). However, it should be stressed that other less known mechanisms may be involved when postmortem muscle tries vainly to reinstate homeostasis (Ferguson and Gerrard, 2014). Consequently, they can reasonably be expected to contribute to abnormal meat quality.

Weather

The incidence of dark cutting is season influenced. Exposure of animals to heat or cold stress can affect intake and metabolic rate. Scanga et al. (1998) have shown the adverse effect of hot seasons on the incidence of dark cutting beef. They noted that incidence of dark cutting beef increased significantly during hot season compared to cooler season. Prolonged heat stress triggers the animal response and behavior, which can impair physiological functions and energy balance. Hormonal activity during heat stress has been shown to promote energy catabolism to the detriment of energy storage mechanisms (Nelson and Drazen, 2000). Adrenergic response to heat stress stimulates muscle glycogenolysis. Heat stress causes physiological distress that drives the use of glucose and gluconeogenic precursors as fuel (Kadim et al., 2004). It has been suggested that under heat stress, lipids seem spared while carbohydrate metabolism is altered to increase production and metabolic oxidation (Baumgard and Rhoads Jr., 2013). Under these conditions, hepatic glucose increases as a result of an increase in both glycogenolysis and gluconeogenesis (Baumrgard and Rhoads Jr., 2013). Baumgard and Rhoads Jr. (2013) believed that the increase in hepatic glucose production is related to an increase in insulin production in heat stressed cattle.

They also suggested that hepatic glucose production in stressed lactating dairy cows is similar to that of unstressed counterparts but preferentially utilized in processes other than milk production.

It appears that carbohydrate metabolism and insulin homeostasis are part of heat stress management mechanisms. Slimen et al. (2016) concluded that heat stressed cattle enter into a negative energy balance state due to reduced feed intake and increased glucose utilization. It is likely contributing to the depletion of glycogen stores before slaughter and could result in high ultimate pH meat.

An increase of dark cutters in autumn has been noticed as cold and wet weather comes along with a reduction in feed availability (Tarrant, 1989). During cold weather, energy expenditure for heat production increases. Oxygen consumption and heat production are enhanced by shivering, which can lower glycogen levels. Also, heat production for body temperature maintenance may contribute to hinder energy storage mechanisms to the profit of energy expenditure. The underlining mechanism has been suggested to be the response of muscle to an increase in adrenergic hormones under cold conditions (Tarrant, 1989). However, these mechanisms and their relation to glycogen depletion and dark cutting condition are yet to be fully understood.

Implant use and management

The aggressive and inappropriate use of growth promoting implants such as beta-agonists may contribute to the incidence of dark cutting beef (Tarrant, 1989; Grandin, 1980). These compounds influence nutrient repartition by their catecholamine like action. Their action results in a leaner carcass. However, Tarrant (1989) suggested that their adrenergic activity may lead to glycogen stores depletion thereby leading to the incidence of dark cutters. Implant management plays a significant role in the incidence of dark cutting beef. Scanga et al. (1998) found that implant selection and time of reimplanting before slaughter influenced dark cutting incidence. These

authors reported that the use of a combination of androgen an estrogen as implant and reimplant increased the incidence of dark cutters. They further showed that holding cattle more 100 d post reimplant decreased the incidence of dark cutters by 38 and 69% for heifer and steers respectively. It is unclear how the implants and their management impair glycogen depletion and other relevant mechanisms leading to the dark cutting condition.

Physical activity and exhaustion

Evidence of the increase in high pH meats has been shown as related to physical stress. Tarrant (1989) underlined the significance of physical activity as a potential cause of dark cutting meat. Muscle contraction mobilizes rapidly energy stored as glycogen to meet energy requirements. Previous research has proven the increase in caloric expenditure (on top of maintenance energy) during physical work is almost entirely due to skeletal muscle contraction. Muscle contraction requires ATP metabolism under aerobic and then anaerobic conditions. In the long run, physical stress may result in glycogen exhaustion. Conversely to resting muscles, muscles required for physical work utilize extensively the glycogen stores and have been shown to exhibit more dark cutters (Bergstrom and Hultman, 1966). Tarrant and Sherington (1980) suggested that mounting and aggressive behavior sustained for long time could result in glycogen exhaustion and subsequently in dark cutting meat.

Muscle type

Muscles are composed, at the structural level, of slow twitch (type I) and fast twitch (type II) fibers. Type I muscle fibers are recruited during a low intensive but enduring activity whereas type II counterparts are mobilized during short and intensive physical activity. The response of muscle fibers types to stress vary greatly according to the muscle metabolic profile. For example, young bulls exhibited greater glycogen exhaustion in fast twitch muscle fibers (Tarrant, 1989). Tarrant

showed that for chronic stress induced by adrenaline administration, slow twitch fibers are more glycogen depleted. For instance, long transportation to slaughterhouses has such an effect. The degree of glycogen depletion will depend on the rate of glycogen resynthesis prior to slaughter (Davie et al., 2001). Loading and unloading, long periods of standing, mixing unfamiliar animals, or restless behavior impact glycogen depletion thereby increasing the incidence of dark colored beef.

Psychological stress

Psychological stress factors can play a significant role in the incidence of dark cutting in beef. Dantzer and Mormede (1981) reported that psychological factors have effects similar to that of physical stress factors. Psychological factors elicit adrenergic hormone release. This results in glycogen catabolism. Under prolonged stress exposure, the animal exhausts its glycogen reserves, which can lead to the development of high ultimate pH meat. For example, the authors found that pigs exposed to a new environment exhibited a similar change in plasma corticosteroids as their counterparts exposed to inescapable electric shock. A review by Grandin (1980) suggested that animals are seriously stressed by uncommon environments. Preslaughter environments the animals deemed dicey cause anxiety and increased adrenergic hormone responses (DiGiusto et al., 1977). For instance, animals arriving at abattoir pens are stressed by this new environment. The nature of noises, the design of the pens, and people handling those animals have all a stressful impact on the animals. Grandin (1980) reported that pre-slaughter handling is one the most important factors to mitigate the effects of stress and the incidence of dark cutting meat.

Tenderness and tenderization

Tenderness is on organoleptic trait that is consistently ranked atop eating satisfaction characteristics by consumers along with juiciness and flavor. It has been established that

tenderness drives consumers' willingness to pay a premium for products that are guaranteed tender (Miller et al., 2001). Variability in tenderness and toughness affects consumer acceptability of meat. Inconsistencies in tenderness remain one of the most serious problems facing the beef industry. There is a significant body of work that suggests beef muscles differ in tenderness and their response to postmortem tenderization. Tenderness and tenderization are influenced by many factors: integrity and degradation of actomyosin, composition and connective tissue or background effect, and the lubrication or bulk density effect. Variability in tenderness between muscles can also be explained by differences in muscle characteristics due to breed, age, gender, feeding management, and muscle type. Different locations on the carcass have different muscle profiles (Rhee et al., 2004) and metabolic rates (Sears et al., 2005). The composition of muscle varies depending on its location, anatomy, and function. Each muscle executes different functions in the living animal. It has been shown that support muscles are more tender than locomotive muscles. For example, locomotive muscles such as *biceps femoris* and *semimembranosus* are tougher than the supportive muscle psoas major (Koohmaraie et al., 2002). The contribution of each of the different factors to meat tenderness is variable and is influenced by pre-harvest, peri-harvest, as well as post-harvest management processes (Tatum, 2006).

Different muscles vary in protein composition. Muscles are composed of sarcoplasmic, myofibrillar and stromal proteins. Within a muscle, muscle cells or muscle fibers are made of sarcomeres known as contractile units. Muscle fibers differ within and between muscles. Red muscle fibers or type 1 muscle fibers predominantly found in support muscles such as the *Psoas major* (PM) have been associated with a more tender meat (Crouse et al., 1991; Koohmaraie et al., 2002). Type II or white muscle fibers that predominate in locomotive muscles are associated with tougher meat. The differences in tenderness between the two types of muscles may be imparted by

differences in diameter (type I has smaller diameter), lipid and myoglobin content (bulk density effect, type I and type IIA), and metabolic pathway (oxidative for type I and type IIA, and glycolytic for type IIB). Yet, a strong relationship of fiber type to tenderness is not unquestionably established and the nature of the weak association so far demonstrated is complex.

The temperature and pH decline in muscles also vary depending on the location and type of muscle. Susceptibility to cold induced toughening is fiber type dependent (Devine et al., 1984). For instance, red muscle fibers have been found to have greater susceptibility to cold toughening than white muscle fibers ones. There is evidence that different parts of a same muscle may vary in response to cold shortening and aging. Muscle fibers located in the innermost parts of the muscle tend to chill more slowly than outermost parts of the muscle. The difference in chilling rate creates a differential metabolism that may result in differential shortening and tenderness.

Sarcomere length has been associated with tenderness. During the conversion of muscle to meat, when energy reserves are depleted, the muscle enters rigor mortis and shortens. As ATP depletes in the muscle, actomyosin bonds are progressively and permanently formed. This results in sarcomere shortening that is associated with toughness in meat. At the completion of rigor mortis, the muscle is at its highest toughness point. Shortening after rigor completion may attain 30 to 60% of the original muscle length depending on environmental conditions such as the temperature and rate of chilling, metabolic activity, fiber type, etc. Innermost muscles that chill slowly than superficial muscles will have a lesser degree of shortening. Many studies have shown that the degree of sarcomere shortening is negatively associated with tenderness. A higher degree of sarcomere shortening results in tougher meat. The degree of sarcomere shortening is dependent upon postmortem management processes. A proper chilling rate and an adequate electrical

stimulation could counteract the effects of temperature - induced shortening and result in improved tenderness.

Structural muscle integrity and or degradation varies between muscles. Kanda et al. (1977) showed that calcium binding ability of the sarcoplasmic reticulum decreases at temperatures below 10°C when pH falls below 6.6. The destabilization of sarcoplasmic reticulum means a release of calcium, which may induce contraction. The main source of protein degradation in postmortem muscle has been suggested to be of enzymatic origin. More specifically, the calpain system (mu and m calpains and calpastatin) has been suggested to be the main driver of postmortem muscle proteolysis (Koohmaraie and Geesink, 2006, Geesink et al., 2006).

Calpain activity starts early postmortem and targets cytoskeletal muscles (titin, nebulin, desmin, costameres, etc). Calpastatin is the inherent inhibitor of calpains. Different muscles have different enzymatic profile. For example, callipyge lambs and Brahman cattle that have high calpastatin have tougher meat than normal lamb or *Bos Taurus* cattle (Koohmaraie et al., 2002). Other proteolytic enzymes such as caspases, cathepsins, and proteasomes have little to no contribution to postmortem tenderization during aging.

The response of different muscles to postmortem degradation is muscle dependent (Ouali & Talmant in 1990). Ouali and Talmant (1990) showed that the loin muscle and masseter have different calpain and calpastatin profile and activity, hence their variation in tenderness and tenderization. Aging or conditioning is the process whereby muscle meats are kept at – 1 to 4°C for a predetermined time postmortem to allow rigor resolution and tenderization of the meat.

Connective toughness is dependent upon amount and solubility of the connective tissue content of muscles. Collagen and elastin help maintain different muscles attached to the skeleton. Hence, the contribution of these components in muscle toughness increases in locomotion muscles and

decreases in support muscles. For example, the tenderloin (PM) has lower connective content than semimembranosus (Koohmaraie et al., 2002, Stolowski et al., 2006). Postmortem proteolysis has little to no effect on connective tissue up to 10 days postmortem (Koohmaraie et al., 2002).

Lastly, fat content (marbling) of muscle may influence tenderness (Moody et al., 1970, Li et al., 2006). Fat molecules provide lubrication and bulk density thereby contributing to tenderness perceptions. It has been suggested that intramuscular fat may affect content and strength of connective tissue (Moody et al., 1970). However, Vierck et al. (2018) found no evidence to support the effects of marbling on connective tissue toughness. Furthermore, Jones et al. (1991) determined that the degree of marbling did not impact tenderness scores and WBSF values.

Effects of carcass size on meat quality

The average carcass weight has increased over the last 20 years in the US as evidenced by the National Beef Quality Audits (Boykin et al., 2017a). Improved efficiency caused by reproductive management techniques (Dahlen et al., 2014), genetic and nutritional improvements (White et al., 2015), and disease control (Sneeringer et al., 2015) had allowed beef producers to produce more beef in times when cattle numbers had plummeted or stayed level. However, increased carcass sizes and mass have caused challenges for producers and distributors of beef (Maples et al., 2018). More specifically, there is a discrepancy between new carcass dimensions and infrastructural and management procedures primarily tailored to accommodate lighter and smaller carcasses (Maddock, 2015). Simultaneously, inconsistencies in tenderness, purge loss, and color remain currently a challenge for the industry (Bekhit et al., 2014). Carcass size can affect meat quality through multiple mechanisms. It is reasonable to assume that carcasses of different sizes will respond differently to postmortem processes and practices to enhance meat quality (Agbeniga and Webb, 2018).

Carcass composition can influence chilling rate through fat cover and marbling. Carcasses that have greater subcutaneous fat thickness, which could serve as insulation and slow down chilling rates, will chill more slowly. A relationship between subcutaneous fat thickness and rate of temperature decline in muscles has been reported in beef, lamb, and pork studies (Aalhus et al., 2001, Agbeniga and Webb, 2018). Thicker fat covers are related to slower chilling rate and faster pH decline. As, mentioned earlier a slower rate of chilling is accompanied by faster pH decline. A very fast pH decline coupled with high temperatures cause protein denaturation and a paler meat color (Kim et al., 2014). In lamb and beef, such conditions may be favorable as they tend to prevent cold shortening and improve grade parameters, especially a brighter and youthful loin color.

Moreover, greater marbling affects chilling and pH decline and improves grade scores in beef and lamb, flavor, tenderness, and juiciness in all species (Picard et al., 2018). Marbling is beneficial to flavor because the degradation of lipids during cooking along with Maillard reaction products develop the characteristic flavor of meats. In fresh and cooked meats, lipid oxidation products (due increased fat content) as major deteriorative effectors of meat flavors. Hence, larger carcasses may be more susceptible to such processes.

Marbling affects tenderness through bulk density effect, insulation effect, flavor and juiciness effect (perception), and connective tissue or background effect (Wheeler et al., 1994, Thompson, 2002). Fat is less dense than water, and the deposition of fat molecules not only makes the muscle easy to chew but also weakens the connective tissue structure and strength. Heavy carcasses, with high marbling scores tend to perform better with regard to tenderness and tenderization process (Stolowski et al., 2006). They are more tender and tenderize more quickly than leaner carcasses. Marbled meats appear to be juicier and more flavorful and therefore seem to be more tender.

The slow rate of chilling due to fat insulation (both from subcutaneous and intramuscular fat) may favor temperature conditions that allow lower muscle shortening (more tender) and enhance proteolytic enzymatic activity (Aalhus et al., 2001). Proteolytic tenderization occurs mainly early postmortem, the first 72 hours or shorter time for monogastrics and birds (Thompson, 2002). In beef and lamb, and even poultry (turkey), electrical stimulation can aid the tenderization process by hastening ATP utilization and rigor onset, speeding up glycolysis, and disrupting the muscle to allow the release of lysosomal enzymes as well as activating the calpain system with the release of calcium (Stolowski et al., 2006).

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CHAPTER II

THE IMPACT OF CARCASS SIZE, RATE OF CHILLING, AND ELECTRICAL STIMULATION ON BEEF POSTMORTEM GLYCOLYSIS AND COLOR

Introduction

Chilling carcasses immediately after slaughter has greatly reduced food safety risks. The removal of heat by carcass refrigeration retards microbial growth and depresses enzymatic activity and metabolic velocity. Although the primary objective of carcass chilling is to limit rapid bacterial proliferation and meat spoilage, its management is crucial for meat quality (Savell et al., 2005). The more rapidly carcasses are chilled, the more protected they are against bacterial growth and spoilage (United States and Marketing, 1971). However, rapid chilling has been associated with the development of cold-induced shortening and subsequent toughening that is detrimental to beef acceptability (Locker, 1985). The extent to which chilling affects meat quality depends on chilling parameters (James, 2002). Appropriate chilling allows carcasses to achieve adequate temperature decline within a reasonable time postmortem, while it prevents toughening issues of meat due to cold or heat shortening (Lawrie, 2006b).

One way to prevent cold shortening is to use electrical stimulation (Calkins et al., 1980), a process that has been extensively used in the beef industry in combination with chilling protocols. The passing of an electrical current through a carcass accelerates glycogen and adenosine triphosphate (ATP) depletion prior to the onset of rigor mortis such that cold induced contractions/shortening are minimized (Savell et al., 2005). Previous research has shown that the

degree of changes induced by electrical stimulation may vary relatively to carcass characteristics and electrical parameters (Devine et al., 2014).

Growing carcass size and increased carcass mass are contributing to issues with beef tenderness, purge loss, and beef muscle color, as a result of an ever-increasing struggle for beef packers to appropriately chill beef carcasses (Solomon et al., 1986). Heavier carcasses require additional time to reach adequate deep tissue temperatures that can ensure safety and quality (Klauer et al., 2018). However, in commercial plants, carcasses are subjected to the same chilling conditions regardless of size and weight. This is due to infrastructures that were at first designed for smaller carcasses and have not undergone significant changes to keep up with carcass size and mass increase over the years (Savell et al., 2005). Consequently, resulting differences in the rate of heat dissipation and pH decline, and rigor onset may be contributing to tenderness inconsistencies and issues with beef color.

Additionally, for foodservice and retail customers of beef, variability in eating quality of beef remains one of the top priority for the beef industry (Martinez et al., 2017). Furthermore, increased fat cover and marbling have been suggested to act as a potential insulating barriers that could reduce the rate of temperature and pH decline, hence influence meat quality (Koohmaraie et al., 1988b, Solomon et al., 1986, Aalhus et al., 2001). However, it appears that current research does not provide sufficient data to answer questions related to variations in the rate of chilling of present beef carcasses and their relation to inconsistencies in beef quality.

Therefore, the objective of this study was to identify the relationship between carcass size, chilling, and electrical stimulation, and its impact on temperature decline, postmortem metabolism, and color of the tenderloin. Moreover, the study sought to develop a model to predict deep and surface temperatures in beef carcasses of varying sizes.

Material and methods

Live Animal Assessment and Carcass Selection

Data collections for this study were conducted at two different commercial processing plants (Plant A and Plant B) in the US. The feedlot of origin, lot number, tag color, sex, arrival time, and average lot weight were recorded for the cattle within identified lots (data not included). Cattle were filmed with a GoPro camera (GoPro Hero 4, GoPro Inc., San Mateo, CA) attached to the overhead walkway in the holding yards. Tag color and lot number served to identify the cattle being recorded. Cattle entrance speed and reaction to a passing researcher walking at a slow pace in the overhead walkway were recorded on the GoPro.

Once cattle were rendered unconscious and exsanguinated, blood samples were immediately taken from the last ten to twelve heads in each identified lot. Blood was collected from the jugular vein immediately following exsanguination. A glucometer (One Touch Ultra 2, Johnson & Johnson, New Brunswick, NJ) and lactate meter (Scout, EKF Diagnostics, Cardiff, UK) were then used to measure blood glucose and lactate levels respectively. Glucometer and lactate meter readings were recorded for each individual animal of the lots identified. An initial hundred and ninety-seven (N=197) live cattle were identified.

GoPro footage of the identified lots was analyzed by a trained third party. The footage was used to evaluate whether cattle were calm or flighty, based on how quickly and how far the animals pulled away from the researcher. Cattle entrance speed was evaluated as either a walk or trot.

Carcass data collection

Hot carcass weights of identified carcasses ranged from 255 to 545 Kg. Of the hundred and ninety-seven (N = 197) live cattle identified, hundred and sixty-two carcasses were utilized to determine the effect of electrical stimulation, chill rate, and carcass size on temperature and pH

decline. The left or right side of each carcass was electrically stimulated (ES) and the opposite side was not stimulated (NES). Electrical stimulation parameters were unique to each packing plant. In Plant B, carcasses sides designated for stimulation moved through the electrical stimulation frame where their middle section made contact with electrode bars delivering a 60 - Hz pulses of varying voltage levels. The voltage increased from 25, to 35, 45, and 55 V. This treatment constitutes the low voltage electrical stimulation (LVES). Carcass sides subject to ES in plant A received in their midsection a high voltage ES (HVES). Hence, in the following text, Plant A will imply Low Voltage ES, LVES; and Plant A, High Voltage ES, HVES. Each pair of sides was assigned to a conventional rate of chilling (CC) or delayed chilling (DC). Both chilling protocols involved spray-chilling. For analysis purposes, carcasses were grouped into heavy and light weight categories based on the average weight of the sample population in each plant. Carcasses with hot carcass weight above or below the average were considered heavy or light weight respectively. The resulting treatment combination of carcass size (heavy = H, light = L), electrical stimulation and chilling provided eight levels for each plant: HDCNES, HDCES, HCCNES, HCCES, LCCNES, LCCES, LDCNES, and LDCES.

Immediately following carcass arrival in coolers, deep tissue and surface temperature were continuously monitored on selected paired sides using a multi-use temperature recorder (Multitrip™ Green, Temprecord International Limited, Auckland, New Zealand) until chilling was completed according to each plant's protocol. Deep tissue temperature was measured by inserting the temperature probe tip (10.5 cm) into the muscle under the haich bone. Surface temperature was taken 1.5 cm under the *Longissimus dorsi* (LD) fat, perpendicular to the muscle, and approximatively 3 cm above the rib cut. Temperature recorders were removed from the carcass sides before they entered sale coolers (26 to 36 h post exsanguination).

The *Longissimus lumborum* (LL), *Psoas major* (PM), and *Semimembranosus* (SM) were used to represent muscles of different glycolytic rates. For each carcass side, 10 g samples were removed at the following time intervals from the LL, PM, and SM: immediately at arrival in the hotbox (45 to 60 min post exsanguination), 6 h, 12 h, and at the completion of chilling before carcasses are moved to sale coolers (18 to 28h postmortem). Similarly, temperature was measured on the SM, PM, and LD using and handheld AquaTuff™ 351 Wrap&Stow™ thermometer equipped with a DuraNeedle probe (Cooper Atkins Corp., Middlefield, CT) and inserted into the geometric center of the muscle, 5 cm deep, and perpendicular to the muscle surface. Muscle samples were immediately frozen in dry ice (carbon dioxide) to stop glycolytic processes and pH decline. The samples remained frozen (- 62.22°C) until further analysis.

Determination of pH

The pH was determined by the method described by Bendall (1973). Briefly, an approximate 1g of frozen muscle sample (1 ± 0.1 g) was rapidly homogenized (household blender Oster, Boca Raton, FL) in 10 ml of a solution of sodium iodoacetate (5mM) in potassium chloride (150 mM). Sodium iodoacetate stops acidification in meat and prevents further pH decline. The pH of the solution was measured using an electrode and a calibrated tabletop pH meter (Accumet model 13-620-285, Fisher Scien7; Pittsburgh, PA).

Color measurement

Short loins from paired carcass sides were obtained and transported to the Center for Meat Quality and Safety meat laboratory at Colorado State University, where the PM was removed and a 2 cm steaks were retrieved from the butt portion and bloomed for 30 min. A six (6) member semi-trained panel ranked steaks on an 8-point color scale: 1 = light pink, 2 = pinkish red, 3 = reddish pink, 4 = light cherry red, 5 = cherry red, 6 = dark cherry red, 7 = dark red, 8 = very dark

red, and 9 = purplish red. Following panel color assessment, instrumental color measurements (CIE L*a*b*) were made using a portable spectrophotometer equipped with a 6-mm aperture, illuminant A, and 10° standard observer (MiniScan EZ; HunterLab Associates Laboratory, Reston, VA). The color was measured at three random locations on the bloomed surface of PM steaks and averaged for each steak.

Glycogen measurement

A total of 16 animals were subsampled, 8 from each chilling group for glycogen assessment in plant B. Samples from the LL were used to estimate postmortem glycogen depletion. Glycogen measurement was completed using the BioAssay System's glycogen kit according to the manufacturer's protocol. Samples preparation was conducted according to the method described by Murat and Serfaty (1974) with a slight modification. Briefly, frozen samples were powdered in liquid nitrogen. Powdered tissue samples (0.25 g) were homogenized in 5 mL ice cold buffer (2.5g/L sodium fluoride in 25mM sodium citrate, pH 4.2) and centrifuged at 14,000 x g for 5 minutes. For the assay, 10 uL of the clear supernatant was used. Glycogen levels in samples were determined by the colorimetric procedures described by the kit's manufacturer (EnzyChrom™ Glycogen Assay Kit; Cat# E2GN-100).

Statistical analysis

Blood sample measurements, along with cattle temperament scores, were analyzed using a paired t - test (SAS Institute Inc. 2013, Cary, NC). Effects of carcass size (H vs. L), chilling rate (CC vs. DC), and electrical stimulation (ES vs. NES) on temperature and pH decline for individual muscles (PM, SM, LL), objective color parameters and subjective color scores, and glycogen levels were determined using mixed models. Except for the dependent variables subjective color scores and objective color parameters (L*, a*, and b*), the data were analyzed as repeated

measures. The study design was a split-plot design with carcass serving as the whole plot and carcass side as a sub plot. The treatment structure was a 2x2x2 factorial with main effects of carcass size, chilling rate, electrical stimulation, and their interactions with time postmortem included in the model. Animal identity (ID) and electrical stimulation within animal ID (ID*ES) were included as random variables. The MIXED procedure of SAS 9.4 (SAS Institute Inc. 2013, Cary, NC) was used to fit the model. Least squares means and standard errors of the means (SEM) were reported for main effects and significant interactions. The PDIFF option was used to separate means with a significance level set at $P \leq 0.05$. Trends were considered when $0.05 < P \leq 0.08$. The same analyses were performed for data obtained in both plants.

Exponential decay model

Mathematical derivation with respect to a variable gives the slope at a particular point on a curve. If the variable is time, the classical derivative gives the time rate of change at the particular point in time. To fit deep tissue and surface temperature of beef carcasses, exponential decay models were developed to create temperature curves using the following packages in R: ggplot2 (Wickham, 2016), and dplyr (Wickham et al., 2017). The Self-Starting Nls Asymptotic Regression (SSasymp) function in R (Bates and Chambers, 1992) was utilized to fit self-starting nonlinear exponential decay models to least squares means of temperature for any combination of carcass size, chilling rate, electrical stimulation and time postmortem following the equation below:

$$T = T_a + (T_0 - T_a)\, e^{-\lambda t}$$

where T is the temperature at time t ; T_a , $(T_0 - T_a)$, and λ represent the model parameters. The parameter λ represents the rate of chilling; T_a is the asymptote and represents the value of temperature when time tends to infinity (ambient temperature); and $(T_0 - T_a)$ the difference between initial temperature of the carcass (T_0) and the ambient temperature (T_a).

Least squares means for deep tissue and surface temperature of the combination of carcass size (H vs. L), chilling rate (CC vs. DC), and electrical stimulation (ES vs. NES) were plotted against time and presented in Figure 1 and 2 for plant A and B respectively. The plots show both deep tissue and surface temperature curves follow an exponential decay pattern and evidence the geometrical behavior of observed temperature decline of beef carcasses.

Results

Animal live observations and blood characteristics

Table 1 summarizes blood characteristics of selected cattle. There was no difference ($P > 0.05$) between calm and flighty.

Travel distances from feedlot to the plant were categorized into “near” (0-19km), “mid” (20-97km), “far” (98-183km), and “furthest” (184 or more km). Cattle that travelled “near” (199.8 mg/dL) and “mid” (193.1 mg/dL) did not differ ($P > 0.05$) in glucose levels from one another. However, cattle that traveled “far” (227.2 mg/dL) and “furthest” (164.1 mg/dL) were different ($P < 0.05$) from one another and from the “near” and “mid” cattle. The lactate levels for the cattle traveling “near”, “mid”, and “far” were not statically different from one another; however, cattle traveling the “furthest” distance had lower ($P < 0.05$) lactate levels than the “near” and “mid” cattle but not the cattle travelling “far”.

Lairage time (data not shown) was categorized into short (less than 5 hours) or long (more than 5 hours) and appeared ($P < 0.06$) to influence glucose levels but not lactate concentrations. Cattle in lairage for less than 5 hours tended to exhibit higher glucose levels (208.53 mg/dL vs. 185.38 mg/dL) compared to cattle in long lairage. The cattle exiting the pen with a “walk” pace had an average glucose level of 213.1 mg/dL compared to the cattle that had a pace of “trot” with an average glucose level of 186.8 mg/dL ($P < 0.05$). The cattle that had a pace of “walk” also had a

higher average lactate level, 12.7 mmol/L, compared to the “trot” cattle that had an average lactate level of 11.5 mmol/L ($P < 0.05$).

The average levels of glucose and lactate were similar (195.7 mg/dL vs. 203.8 mg/dL and 12.5 mmol/L vs. 11.6 mmol/L) for plant A and B respectively. Sex did not ($P > 0.05$) influence blood parameters.

Postmortem biochemistry

Temperature decline in different muscles

Temperature decline in the LL, PM, and SM in plant A and plant B are shown in Tables 4 and 7 respectively. There was a weight category x time postmortem interaction ($P < 0.05$) for LL and PM in both plants. Light weight carcasses had a faster ($P < 0.05$) temperature fall than heavy weight carcasses. The LL of light weight carcasses dropped on average a degree faster than that of heavy weight counterparts between the initial temperature and 12 hours temperature. Temperature fall was not different ($P > 0.05$) thereafter. Similar results were observed in the PM. However, the drop in temperature between the initial time and 6 hours postmortem was on average 2 degrees faster in plant A. This is more likely due to the environmental temperatures of the coolers in plant A that were on average 2 to 3 degrees lower than temperatures in the coolers of plant B (data not shown). There was a significant ($P < 0.05$) weight category*time interaction for the SM in plant A. Temperature decline in the SM was 2 degrees faster ($P < 0.05$) in light weight carcasses than in heavy weight carcasses throughout the chilling period. For all muscle types, the heat dissipation rate was greatest within the first 6 hours postmortem for both plants. There was on average 15 degrees removed within the first 6 hours compared to 9 degrees removed between 6 and 12 hours and 12 and the final time postmortem (28 to 36 hour). Although a two-way interaction ($P < 0.05$)

between chilling rate and time postmortem was detected in the LL in plant A, the rate of temperature decline was similar between the two chilling groups.

The rate of pH decline

Before to electrical stimulation and chilling, the LL of 31 and 36 carcass sides respectively collected at plant A and plant B were sampled for an initial pH. The mean pH in plant A was 6.38 and ranged from 6.12 to 6.71. In plant B, the mean pH was 6.58 with a range of 6.15 to 7.02. The change in pH is shown in tables 5, 6 and 8. In plant A, a weight category x chill rate x electrical stimulation x time postmortem was detected for LL pH decline (Table 5). In such case, it is challenging to distinguish the factor that has the most significant influence on pH decline. In general, pH fall was 0.1 unit faster in stimulated sides than in non-stimulated over the first 12 hours regardless of the chilling rate and carcass size. It is worth noticing that initial pH, measured approximately within an hour of exsanguination was lower than values reported previously and varied from 5.58 to 6.56 (data not shown). Also, there were weight category x chill rate x time ($P < 0.01$) and chill rate x time ($P < 0.01$) interactions for pH fall in PM Table and SM, respectively (Table 6). The PM of heavy weight carcasses under conventional chilling had the greatest fall in pH (0.2 pH units) between the initial time and 6 h postmortem, followed by that of light weight carcasses under delay chilling (0.09 pH units). The initial pH in the PM varied from 5.4 to 6.41. In the SM, pH drop was faster (0.53 pH units, $P < 0.01$) in DC carcasses than in CC carcasses (0.38) within the first 6 hours postmortem. The extent of pH decline in the SM of both groups was similar over the chilling period (0.81 vs 0.83 pH units).

In plant B, an interaction ($P < 0.05$) between carcass size (weight category) and time postmortem was detected for all three muscle types (Table 8). In general, heavy weight carcasses showed a most rapid pH decline within 12 hours of exsanguination. Initial pH was lower in heavy

weight carcasses across all muscles. The LL and PM reached ultimate pH by 12 h postmortem whereas SM reached ultimate pH between 12 h and the final chilling time. The PM reached the ultimate pH within 6 h postmortem.

Glycogen depletion

There was a significant effect of electrical stimulation ($P < 0.01$), and time postmortem ($P < 0.0001$) on postmortem glycogen depletion. Glycogen levels in stimulated sides (170.92 ± 3.85 µg/mL) were lower ($P < 0.01$) than that measured in non-stimulated sides (181.58 ± 3.85 µg/mL). More specifically, depletion of glycogen in stimulated sides was faster than in non-stimulated counterparts. Glycogen levels were not different ($P > 0.05$) between light (175.59 ± 15.57 µg/mL) and heavy weight carcasses (176.97 ± 15.57 µg/mL). The depletion of glycogen in LL was parallel to that of pH. Pearson correlations were computed to assess the relationship between glycogen depletion and pH decline.

Subjective color of the PM

Electrical stimulation has a significant ($P < 0.05$) effect on panel color scores in both plants. More specifically, electrically stimulated sides were redder (5.35 and 5.12) than non-stimulated sides (4.78 and 4.88) in plant A and plant B respectively. In plant A, 82% of stimulated sides were ranked light cherry red or cherry red while only 66% of non-stimulated sides were ranked as such. There were twice as much non-stimulated sides (34%) classified as dark red or very dark red compared to stimulated sides (17%). In plant B, 62% of stimulated vs 55% of non-stimulated sides were classified as bright cherry red or cherry red. There were fewer (30% vs. 41%) stimulated sides ranked as dark or very dark red. Regardless of plant, carcass size and chilling rate did not influence subjective color scores of the PM.

Objective PM color measurement

In plant A, redness (a*) was not affected ($P > 0.05$) by treatment factors. There was a significant interaction ($P < 0.05$) between carcass size and chilling rate for yellowness (b* values). Delay chilling improved b* values ($P < 0.05$) for light weight carcasses (18.23) when compared to heavy weight carcasses (16.40). An interaction ($P < 0.05$) between carcass size and electrical stimulation was detected for lightness (L*). Electrical stimulation increased ($P < 0.05$) L* values in light weight carcasses (54.66 for stimulated sides vs 49.94 for non-stimulated sides). Lightness (L*) of heavy weight carcasses was not affected by electrical stimulation.

In plant B, heavy weight carcasses were redder (higher a* value, $P < 0.05$) than light weight carcasses. Also, carcass size influenced b* values, with heavy weight carcasses having more yellow color ($P < 0.05$) than light weight carcasses. Lightness was not affected by the treatment factors in plant B.

Deep tissue and surface temperature decline exponential decay models

Model selection

Figures 3 and 4 show predicted lines plotted over the observed data for each treatment group in plant A and B respectively. The regression curves allowed estimation of the cooling rates or constants λ. It appears that the temperature curves show some noise and discrepancies with the data that need to be addressed. During the first hour, deep tissue temperature appeared to increase up to a certain level where it plateaued and decreased thereafter for all treatment groups. This initial increase in temperature may correspond to an equilibration phase where a certain number of factors play a significant role. The first factor to consider could be the increase in temperature generated by postmortem glycolysis. According to Meis (2001), beef carcass temperature could raise by 2.1 to 2.7°C above body temperature during the first hours following death. This increase

in temperature is a result of postmortem hydrolysis of ATP, which is responsible for an enthalpy release of approximately ΔH_{ATP} = 134 kJ/mol Pi. Theoretically, the difference in temperature between the body being cooled and its environment is small, and the rate of temperature exchange is constant. In consequence, the model should perfectly fit the experimental data provided the conditions above are met. In practice, this is almost impossible to achieve in most temperature decline models (Davidzon, 2012). Given the rate of heat transfer depends on the mass, surface, and specific conductivity of the material, the presence of a thermal gradient (Mondol et al., 2018) in such biological systems as beef carcasses hinders the ability of a mathematical model to fit the experimental data.

Heat removal proceeds from the surface of carcasses to the innermost parts. In practice, there are significant variations in biochemical composition of beef carcasses as one goes from the external parts to the most inner parts. The differences observed between the fitted lines for deep tissue temperature (figures 2 and 4) and surface temperature (5 and 7) are more likely influenced by local differences in proximate composition. At the surface, fat cover may play a more significant role while the type and composition of the muscle may play a more significant role in deeper tissues. Consequently, because of the inherent differences in heat conductivity and emissivity, density, and specific heat of different tissue components (Jiménez-Muñoz et al., 2017) the variation in tissue composition (proximate) could be contributing to alter the rate of heat transfer (at least to a certain degree) and induce a temperature gradient between locations (surface vs deep) and possibly among the different treatment groups. This is in agreement with Kuffi et al. (2016) who found significant differences in local coefficients of transfer at the surface (2 cm beneath fat) and deep tissue (18 cm deep) of beef carcasses. At the surface of the carcass, the heat transfer is associated with a significant mass transfer subsequent to evaporation (Savell et al.,

2005) during the first hours of cooling. Therefore, the rate of temperature change at the surfaces may be more affected by evaporation and radiation than that of deep tissue temperature early in the chilling process whereas the latter may be more influenced by metabolic heat production. Kuffi et al. (2016) showed that metabolic heat production at the beginning of the cooling process seems to exceed the amount being diffused towards the surface.

Moreover, significant afflux of hotter/warmer carcasses in blast chillers in early postmortem may result in an increase in environmental temperature as evidenced by the environmental temperature data (data not shown). In addition, temperature measurement is a complex process where fluctuations may be due to instrumental errors as well as errors due to operating the temperature loggers (Jiménez-Muñoz et al., 2017). Temperature data from some carcasses were eliminated from the study because of faulty data loggers. The response-time of the instrument during measurement depends on the instrument as well as the specific undergoing measurement (Moggio et al., 2017). For example, data acquisition by a temperature logger depends on the feature of the instrument (sensitivity or lag time) and the equilibrium between the logger and the carcass. This could also contribute to the noise observed in the experimental data.

The fitted curves for the surface temperature showed some discrepancies with the actual temperature data. Important environmental temperature fluctuations occur regularly in blast chillers where carcass turnover is high. To minimize evaporation and carcass shrink, warmer carcasses are sometimes chilled in alternate rails adjacent to cooler carcasses. It is not unexpected that surface temperature is more affected by the fluctuations inherent to the current consist of chilling practices.

Rate of temperature decline in treatment groups

The R squares of the model (Tables 9, 11, 13, 15) suggest that the fitted lines explained most of the variability of the data. The self-starting Nls asymptotic regression has an initial attribute that allowed an evaluation of initial estimates of the parameters Ta, (T0 – Ta), and λ for each temperature data set. The selftStart function of SSasymp in R evaluates the regression function and its gradients. The advantage of using an asymptotic exponential decay model to fit temperature decline in beef carcasses is that it is one of simplest models that describe best the asymptotic and Newtonian behavior of carcass cooling as previously explained.

Tables 9, 11, 13, and 15 show parameter estimates for the fitted model. The rates of temperature decline in each group as represented by λ were used to compare the different groups to the conventionally chilled non-stimulated light weight carcass sides (LCCNES, considered as a reference group) for both deep and surface temperatures (Tables 10, 12, 14 and 16). A greater cooling constant (λ) for a treatment group means that temperature decline in that group was faster than that of the reference group. In plant B, the rates of cooling for deep tissue temperature in the light group were similar ($P > 0.05$) to that of the reference group except for the LDCNES (light weight carcasses in delay chilling group, $P < 0.05$). In the heavy weight group, HCCNES and HDCES had rates of cooling that were not different ($P > 0.05$) to that of the reference group. The HDCNES and HCCES treatment groups had rates of chilling that were significantly different than that of the reference group. The rate of deep tissue chilling was smaller in heavy weight carcasses when compared to the reference group. This means that heavy weight carcasses chilled at a slower rate than the reference group. For surface temperature, only the LDCNES group had a different rate of cooling ($P < 0.05$). In plant A (Table 16), all treatment groups cool at a different rate than the reference group for deep tissue temperature. For surface temperature decline in plant A, the

chilling rates of all groups were different ($P < 0.0$) from the reference group except for the LDCES groups. Similar to the results in plant B, the rates of cooling were much smaller in heavy weight carcasses compared to the reference. More specifically, the rate of chilling in delay chilled heavy weight carcasses is 2 to 3 times smaller than that of heavy weight carcasses chilled under conventional chilling and 3 to 5 times smaller than that of the reference group.

Discrepancies between different models developed to fit temperature decline in livestock species and actual experimental data behavior highlight real difficulties in using mathematical modeling to explain natural phenomena (Bruce, 2004, van de Ven et al., 2014). A variety of models have been developed with relative successes since many variables (carcass conformation, air characteristics, spray) come into play. These variables increase the level of challenges relative to determining unbiased estimates that could be used under multiple processing conditions (Kuffi et al., 2016, Bruce, 2004, van de Ven et al., 2014). The purpose of modeling temperature decline for livestock species is that unbiased and reliable estimates and predictions of chilling conditions could be made with high confidence with respect to individual or groups of carcasses. A good model should be versatile enough to accommodate different sets of data and allow extrapolations beyond the data. The model herein developed is a simple and appropriate fit for temperature decline in stimulated and non-stimulated heavy and light weight carcasses under two different cooling regimes.

The major shortcomings of the modeling approach used in the present study at the moment are relative to the fact that average temperatures were used and carcass to carcass variability was not accounted for. The second aspect that may need further investigation is testing the model against a subset and determine the goodness of fit for individual carcasses. Another step would be to

include environmental temperatures and fat cover in the model and determine its fit to the observed data.

Modeling temperature decline is a complex phenomenon that has garnered the attention of many scientists since Sir Isaac Newton formalized his observations into the now renown law that bears his name (Ugo, 2011). O'Connell captured the debate in this statement: "Newton's law of cooling is one of those empirical statements about natural phenomena that should not work, but does." (Mondol et al., 2018). Beef carcasses are complex biological systems, and this adds a different level of complexity to a phenomenon that is already complex. The cooling of beef carcasses is influenced by various flow variables (convection, conduction, radiation, metabolic heat production) and thermophysical properties of the carcass (conductivity, emissivity), the cooling air, and the cooling room boundaries (types of walls).

Discussion

Blood glucose and lactate have been used in this study as secondary indicators of stress. Plasma characteristics showed cattle that traveled the furthest distance had the lowest levels of glucose but high lactate concentrations. This agrees with Warner et al. (2007) who reported a spike in blood lactate concentrations in cattle under high stress. Cattle in the latter study were acute stressed with electric prodders. The levels of blood lactate in the present study were relatively lower than that reported by Warner et al. (2007) possibly because cattle in this study were relatively under low stress. Lactate levels were similar to that reported by Cottrell et al. (2008) and Pighin et al. (2014) in lambs. Although lactate concentrations in the flighty temperament group and trot group were expected to increase as a response to stress, they decreased. This is more likely due to animal variability and to the fact that the level of stress undergone by the cattle may be low. Previous investigations using blood characteristics as an indicator of pre-slaughter stress have reported

various effects of stress on plasma glucose and lactate, more specifically an increase in blood lactate (Pighin et al., 2014, Warner et al., 2007, Edwards et al., 2010, Hambrecht et al., 2004). Hambrecht et al. (2004) suggested that the increase in lactate concentrations could serve as a sensitive tool to assess handling stress in swine. Pre-harvest factors that induce physical or physiological stress on cattle have been shown to impact meat quality and produce dark cutting beef.

Pre-slaughter stress, whether acute or chronic, elicits a rapid breakdown of glycogen in response to an activation of the nervous or endocrine system via catecholamines and glucocorticoids release (Dantzer, 1994, Grandin, 2014, Tarrant, 1989). Low levels of glycogen antemortem could result in an insufficient postmortem pH decline. The subsequent meat has high pH, retains more water, appears darker in color, and is unappealing to the consumer (Tarrant, 1989, Ferguson and Warner, 2008, Viljoen et al., 2002, Holdstock et al., 2014).

Exhaustion of muscle glycogen due to chronic stress before harvest has been recognized as a major cause of dark cutting beef. The sample population in the present study appeared to be subjected to mild levels of physiological and physical stress, which could explain the marginal impact of live observations on blood lactate concentrations and meat color.

The relationship between temperature and pH decline is an important parameter in the monitoring and control of postmortem processes to enhance meat quality. The rate and extent of heat dissipation, especially in the early time following animal death, has been studied and its influence on postmortem metabolism has been a subject to numerous investigations (Bruce, 2004, Ferguson et al., 2001). Yet, the continuous increase in carcass size and subsequent tenderness and beef quality challenges warrant more research to better understand its impact. Savell et al. (2005)

suggested that an appropriate chilling of heavier carcasses remains one of the main challenges facing beef processors.

The present study had the potential to provide some insight into the effects of a combination of electrical stimulation and chilling rates on postmortem glycolytic metabolism among the current consist of US cattle. The study highlighted significant differences in the rate of temperature decline and pH decline between heavy and light weight carcasses. More specifically, the muscles of the round (represented by SM) of heavy weight carcasses showed the slowest chilling rates compared to the middle muscles represented by LL and PM. In agreement, Agbeniga and Webb (2018) reported slower temperature decline and faster pH fall rates in heavier beef carcasses compared to lighter ones. The pronounced differences in the rate of chilling and pH decline in heavy weight carcasses may potentially be due to location in the thickest and heaviest section of the carcass, bulk density, marbling, and fat cover. It has been previously suggested that increased mass and/or a combination of fat cover and marbling acting as an insulator may contribute to differences in the rate of chilling and temperature decline in heavier carcasses (Aalhus et al., 2001, Savell et al., 2005, Juárez et al., 2016).

It has been known for decades that the rates of temperature and pH decline greatly influence meat quality. The velocity of postmortem anaerobic degradation of glycogen and glucose resulting in accumulation of lactate and hydrogen protons and the ensuing pH decline depends on muscle temperature. Faster heat removal is associated with a slower pH decline, in which case energy substrates have not been exhausted fast enough for rigor mortis to begin and complete before the muscle reaches temperatures below 10-15°C. If the muscle reaches too low temperatures (< 15°C) prior to rigor onset and completion, cold shortening ensues and tenderness may be compromised (Locker, 1985). Typically, electrical stimulation is used to offset the effect of too fast and/or too

dramatic chilling on cold-induced shortening and subsequent toughening. In this study, it has been shown that electrical stimulation had an interactive effect on pH decline in the LL and glycogen depletion. The rates of glycogen exhaustion and pH decline have both been sped up by the application of electrical stimulation. Previous studies have also shown that electrical stimulation influences the rate and extent of pH decline as well as the rate of glycogen depletion in most muscles of the middle cuts (Eikelenboom et al., 1985, Chrystall and Devine, 1978, Chrystall and Devine, 1985, Chrystall et al., 1980, Chrystall and Hagyard, 1976). Although electrical stimulation has a significant effect on pH decline in the LL, there was no evidence that it did accelerate pH decline in the PM and SM as no significant differences were detected ($P > 0.05$). Most currently used electrical systems have been designed to have a minor if any at all on the muscles of the rounds.

The range of LL pre-electrical stimulation pH in plant A was 6.12 to 6.6.71 with a mean of 6.38. This range captured animal to animal variability. Following stimulation (at the start of chilling), there was a LL pH range of 5.58 to 6.56 with means varying from 6.11 to 6.23 for different combinations of carcass size, chilling rate, and electrical stimulation. The initial decrease in pH averaged 0.4 to 0.5, which is similar to pH fall ranges reported by Chrystall and Devine (1978) for stimulated *Sternomandibularis*. This range of pH fall was observed in both stimulated and non-stimulated sides, suggesting that electrical stimulation may not be solely responsible for the initial drop in pH observed in plant A. Although electrical stimulation interacted with carcass size and chilling rate to affect pH decline in the LL of carcasses in plant A, it is unclear whether its impact was more significant than the two other factors. Similar fall in pH decline was observed in the LL in plant B where electrical stimulation was not significant. This suggests that other factors such as intrinsic biological variability, pre-stimulation muscle pH, time postmortem, and

high carcass temperature may have relatively more significant effects on pH decline than electrical stimulation in the current study. In fact, question arose with respect to the relative contribution of electrical stimulation when temperature is high and could be influencing pH decline. For instance, some researchers questioned whether the fall in pH reported by Chrystall and Devine (1978) was due to high temperatures rather than electrical stimulation (Ferguson et al., 2001). Chrystall and Devine worked on hot-bone muscle (*Sternomandibularis*) kept at 35°C throughout their study. These conditions are clearly not similar to the ones reported in this study. However, it stands to reason that the effect of electrical stimulation on pH decline may be compounded in heavy weight carcasses. Ferguson et al. (2001) argued that though temperature may have played an important role in Chrystall and Devine's (1978) initial pH fall, the effect of electrical stimulation could not be ignored.

The primary objective of beef carcass refrigeration is to reduce carcass temperature, within a reasonable time postmortem, to a level where bacterial growth is controlled or prevented. In the United States, beef processors target 4°C at the surface of the carcass within 24 hours. Most carcasses in this study, whether they were light weight or heavy weight met that requirement for both plants. This has been used in most plants as a critical control point in HACCP plans (Savell et al., 2005). However, deep tissue temperature profiles showed a different pattern. There were significant differences in deep tissue temperature between the two weight groups by 24 h postmortem (data not shown). Deep tissue temperature of most light weight carcasses dropped below 7°C by 24 h while that of heavy weight counterparts remained above 10°C. This is in agreement with Klauer et al. (manuscript in preparation) who found that deep tissue temperature in heavy weight carcasses did not fall below 7°C even after 28 hours of chilling. Although the consensus assumes that surface temperature could suffice as an indicator for food safety, the

present study suggests in corroboration with Klauer et al. that heavy weight carcasses may take longer to achieve adequate internal temperatures necessary to ensure food safety. It is reasonable to think that the additional time carcasses are allowed to chill in sale coolers may limit the risks of having "hotter" carcasses on the fabrication floor. Nevertheless, should chilling periods be reduced, appropriate attention should be paid to heavier carcasses to avoid muscles of the round be chilled inadequately.

The pH of the PM in both plants fell below 6 within an hour postmortem and to its lowest values within 6 h postmortem. Given the PM has been classified as red oxidative muscle, such a significant fall in pH early postmortem is rather unexpected. The kinetics of metabolism are influenced by fiber type composition. Postmortem pH decline has been shown to occur with a faster velocity in glycolytic muscles than in oxidative muscles (Lebret and Guillard, 2005, Listrat et al., 2016). Listrat et al. (2016) suggested however that the relation is not systemic as they observed much lower pH at 45 min in pork PM compared to LD pH. Similarly, Lyon et al. (1983) reported the pH of PM at 1- hour postmortem was much lower than that of *Triceps brachii* which is an intermediary muscle meaning it has both glycolytic and oxidative metabolism. Lower levels of pH within an hour of exsanguination in the PM were also reported by McCollum and Henrickson (1977). A comparative study of postmortem muscle glycolysis between the PM and LD of Korean native cattle resulted in the conclusion that glycolysis proceeds at a higher velocity in PM than in the LD (Kim et al., 2000). The extent of pH decline in PM has been known to be lesser than that of glycolytic muscles as levels of glycogen in muscles are determining for glycolysis. For example, the ultimate pH of the PM reported in the present study was 5.61 while that of the LL and SM were 5.45 and 5.47 respectively.

Electrical stimulation did not affect the rate of pH decline in the PM. It has been previously reported that electrical stimulation did not impact the rate and extent of pH decline in the PM (McCollum and Henrickson, 1977). The muscle fiber composition and metabolic characteristics of the PM, its location in the carcass, the stretching the muscle is subjected to, and its inherent buffering capacity may be contributing to its response to electrical stimulation and postmortem pH fall. In addition, the PM is usually protected by an important quantity of kidney, pelvic, and heart fat. Its location could likely contribute to creating a microclimate that could promote a more rapid postmortem metabolism. In fact, between the initial temperature measurement and 6 h postmortem, there were 1°C and 2°C less removed from the PM than the LL of heavy and light weight carcasses respectively. Considering the surface contact and muscle mass of the PM in comparison the LL, small differences in temperatures could bear different implications provided the rate of heat transfer, conductivity and emissivity of the two types of muscles are different.

In both plants, carcasses were placed on the rails such that the PM was, for the most part, shielded from the chilled spray water. Although the pH values reported in this study were similar to that reported by Agbeniga and Webb (2014), they were in general lower than the values previously reported in the literature for the three types of muscles. This may be due to the methods of determination. Most of the studies that reported higher levels of pH at similar time points postmortem used a method of measurement that involved measuring the pH on the rail with the probe inserted into the muscle (Davey et al., 1976a). The collection of cores for a significant number of carcasses may have hindered the ability to obtains pH measurements that are close to true values and create some noise. In the iodoacetate method of Bendall (1973), additional steps (drill cores, freeze, grind, weight, and homogenize), as well as operators, were likely to introduce some noise in the measurements.

One of the objectives of the current study was to determine whether tenderloin (PM) color variability could be mitigated by the combined effect of electrical stimulation and chilling rate. It appeared that the use of electrical stimulation improved color perception of the tenderloin of beef. The improvement was more noticeable in light weight carcasses. Although objective color coordinates (L*a*b*) were also affected by the treatment factors, it was not possible to clearly identify and establish direct relationships between the variability in color of the tenderloin the factors investigated in the present study.

Lastly, considering carcasses of different sizes and masses are cooled under the same chilling conditions, it makes sense to ponder whether such practices exacerbate issues related to postmortem metabolism and subsequently beef quality. Klauer et al. (2018) reported that as carcass increased heavier carcasses were also larger. They observed that smaller and light weight carcasses were chilled on the same rail as larger carcasses for the same amount of time. Given the rate of postmortem metabolism represented in the current study by the rate of glycogen exhaustion and pH decline is significantly affected by carcass size, tenderness development and the tenderization process of heavy vs light weight carcasses may be affected. It could be argued that segregating carcasses by size/or mass in coolers could help mitigate differences in the rate of cooling and postmortem glycolysis and subsequently enhance beef uniformity.

Conclusion

Increased carcass size and mass is contributing to differences in the rate of chilling and that may have some relevance to aberrant postmortem metabolism. This condition is more likely contributing to beef quality variability and tenderness inconsistencies that could bear some economic implications for the industry. The current industry practices may be exacerbating the issue as carcasses, regardless of their size and mass, are subjected to the same postmortem

processes to enhance quality. Carcasses of different sizes respond with different degrees of efficacy to current postmortem quality management practices, more specifically to chilling and electrical stimulation. This study showed that carcass size influenced tenderness attributes of beef. Provided that further research establishes the economic and scientific relevance of sorting carcasses by size before chilling, it may be possible to mitigate to some extent beef quality inconsistencies by adopting postmortem management processes that take into account carcass variability. Achieving homogeneous rates of chilling represents a pressing challenge and will continue to be a challenge for the meat industry as long as carcass size variability is left out of the chilling management equation.

TABLES AND FIGURES

Table 1: Main effects of transportation, facility location, and behavior measurements on exsanguination blood samples of glucose and lactate in fed-cattle at two geographically different commercial processing facilities (N=197)

	Temperament Score[1] (N = 185)				Travel Distance[2] (N = 197)					
Variable	CALM n = 127	FLIGHTY n = 58	S.E.	*P* - value	NEAR n = 93	MID n = 47	FAR n = 36	FURTHEST n = 21	S.E.	*P* – value
Glucose, mg/dL	209.4	189.9	11.4	0.09	199.8^{a}	193.1^{a}	227.2^{b}	164.1^{c}	15.7	0.05
Lactate, mmol/L	12.5_{a}	11.6_{b}	0.5	0.08	11.8_{c}	11.7_{c}	13.0_{d}	12.1_{cd}	0.7	0.06
	Exit Gait Speed[3] (N = 185)				Facility Location (N = 197)					
	WALK n = 116	TROT n = 69	S.E.	*P* - value	EAST n = 92		WEST n = 105		S.E.	*P* – value
Glucose, mg/dL	213.1^{a}	186.8^{b}	10.8	0.02	203.8		195.7		10.3	0.4
Lactate, mmol/L	12.7^{a}	11.5^{b}	0.5	0.01	12.5_{c}		11.6_{d}		0.5	0.07

[1]Temperament score was evaluated using a walk-back-by test in lairage pens prior to slaughter. Assessments were done via video by a trained, independent observer

[2]Travel distance was determined using information gained from each harvest facility. Distances were split into four categories; NEAR, zero to 19 km, MID, 20 to 97 km, FAR, 98 to 183 km, and FURTHEST, 184 or additional km

[3]Exit Gait Speed was evaluated via video following assessment of temperament. Gait was observed when animals left the assessment pen.

[4]Means with different superscripts (a,b) within the same row differ (P > 0.05)

[5]Means with different subscripts (a,b) within the same row show a tendency to be different (P > 0.08)

Table 2: Effects of carcass size, chilling rate, and electrical stimulation on temperature decline in the Longissimus lumborum (LL), Psoas major (PM), and Semimembranosus (SM) in plant A

Muscle	LL				PM		SM	
	Heavy weight	Light weight	CC[1]	DC	Heavy weight	Light weight	Heavy weight	Light weight
Time1[*2]	36.16dx	39.56dy	37.37dx	38.35dx	32.70dx	37.73dy	37.03dx	39.60dy
Time2[*]	21.14cx	23.42cy	21.90cx	22.65cx	17.26cx	18.59cx	24.01cx	22.85cx
Time3[*]	12.19bx	13.22bx	12.35bx	13.05bx	9.23bx	9.35bx	15.21by	12.60bx
Time4[*]	1.86ax	2.93ax	2.85ax	2.93ax	0.72ax	0.71ax	3.48ay	2.04ax
n	42	40	42	40	42	40	42	40
SEM	0.51		0.31		0.49		0.42	
P- value	< 0.0001		< 0.0001		< 0.0001		< 0.0001	

[1] CC = conventional spray chilling, DC = delay spray chilling

Time1[*2]Time 1 = initial temperature at arrival in blast chillers; Time2 = 6 h of chilling; Time3 = 12 h of chilling; Time4 = final hour of chilling

[a-d] Least squares means within a column with different letters are different ($P < 0.05$)

[x-y] Least squares means within a same row for the same variable and muscle with different letters are different ($P < 0.05$)

Table 3: Effects of carcass size, chilling rate, and electrical stimulation on pH decline in the Longissimus lumborum (LL) in plant A

	Heavy weight				Light weight			
	CC[1]		DC[1]		CC		DC	
	NES[2]	ES[2]	NES	ES	NES	ES	NES	ES
Time1[*3]	6.11cx	6.20cx	6.17cx	6.24cxy	6.23cxy	6.16cx	6.17cx	6.23cx
Time2[*]	5.66bxyz	5.59bxy	5.55bx	5.61bxy	5.67bxyz	5.75byz	5.75bxyz	5.73byz
Time3[*]	5.57axy	5.56bxy	5.50bx	5.53ax	5.63bxy	5.67by	5.68bxy	5.55axy
Time4[*]	5.45ax	5.45ax	5.45ax	5.43ax	5.48ax	5.48ax	5.45ax	5.49ax
n	22	22	20	20	20	20	20	20
SEM	0.041							
P- value	0.021							

[1] CC = conventional spray chilling, DC = delay spray chilling
[2]ES = electrically stimulated; NES = non-stimulated
Time1[*3]Time 1 = initial temperature at arrival in blast chillers; Time2 = 6 h of chilling; Time3 = 12 h of chilling; Time4 = final hour of chilling
[a-d] Least squares means within a column with different letters are different ($P < 0.05$)
[x-y] Least squares means within a same row for the same variable and muscle with different letters are different ($P < 0.05$)

Table 4: Effects of carcass size, chilling rate, and electrical stimulation on pH decline in the Psoas major (PM) and Semimembranosus (SM) in plant A

	PM				SM	
	Heavy weight		Light weight			
	CC[1]	DC	CC	DC	DC	CC
Time1[*2]	5.73bx	5.8bx	5.76bx	5.71bx	6.28dx	6.32dx
Time2[*]	5.68abx	5.60ax	5.64ax	5.66abx	5.75cx	5.94cy
Time3[*]	5.68abx	5.61ax	5.65ax	5.64abx	5.60bx	5.73by
Time4[*]	5.66ax	5.60ax	5.64ax	5.61ax	5.47ax	5.49ax
n	21	21	20	20	41	41
SEM	0.036				0.033	
P – value	0.005				0.002	

[1] CC = conventional spray chilling, DC = delay spray chilling

[2]Time1[*] = initial temperature at arrival in blast chillers; Time2 = 6 h of chilling; Time3 = 12 h of chilling; Time4 = final hour of chilling

[a-d] Least squares means within a column with different letters are different ($P < 0.05$)

[x-y] Least squares means within a same row for the same variable and muscle with different letters are different ($P < 0.05$)

Table 5: Effects of carcass size, chilling rate, and electrical stimulation on temperature in the Longissimus lumborum (LL), Psoas major (PM), and Semimembranosus (SM) in plant B

Muscle	LL		PM		SM
	Heavy weight	Light weight	Heavy weight	Light weight	
Time1[*1]Time1	39.66dx	39.11dx	38.64dx	38.88dx	39.42d
Time2[*]	24.36cy	22.93cx	25.54cy	23.30cx	29.61c
Time3[*]	13.21by	11.26bx	16.16by	13.30bx	18.09b
Time4[*]	4.22ax	3.71ax	4.92ay	3.87ax	5.67a
n	42	40	42	40	82
SEM	0.39		0.42		0.79
P- value	< 0.0001		< 0.0001		0.013

Time1[*] = initial temperature at arrival in blast chillers; Time2 = 6 h of chilling; Time3 = 12 h of chilling; Time4 = final hour of chilling

[a-d] Least squares means within a column with different letters are different ($P < 0.05$)

[x-y] Least squares means within a same row for the same variable and muscle with different letters are different ($P < 0.05$)

Table 6: Effects of carcass size, chilling rate, and electrical stimulation on temperature in the Longissimus lumborum (LL), Psoas major (PM), and Semimembranosus (SM) in plant B

Muscle	LL		PM		SM	
	Heavy weight	Light weight	Heavy weight	Light weight	Heavy weight	Light weight
Time1[*1]Time 1	6.16cx	6.27cy	5.69bx	5.81by	6.34dx	6.42dy
Time2*	5.54bx	5.61bx	5.69bx	5.67ax	5.86cx	6.02cy
Time3*	5.48ax	5.5ax	5.62ax	5.64ax	5.66bx	5.76by
Time4*	5.49abx	5.51ax	5.63ax	5.62ax	5.54ax	5.56ax
n	45	35	45	35	80	
SEM	0.032		0.037		0.035	
P- value	0.038		0.0015		0.05	

Time1* = initial temperature at arrival in blast chillers; Time2 = 6 h of chilling; Time3 = 12 h of chilling; Time4 = final hour of chilling

[a-d] Least squares means within a column with different letters are different ($P < 0.05$)

[x-y] Least squares means within a same row for the same variable and muscle with different letters are different ($P < 0.05$)

Table 7: Parameter estimates of the exponential cooling model fitted to deep tissue temperature in plant B

ID	n	R^2	[1]T_a	$(T_0\text{-}T_a)$	λ
[2]LDCNES	57	0.997	-3.96	47.45	0.054
LCCNES	57	0.995	-3.61	47.74	0.059
LDCES	57	0.997	-3.33	46.64	0.056
LCCES	57	0.995	-4.60	48.38	0.054
HDCNES	57	0.997	-3.54	46.82	0.049
HCCNES	57	0.998	-1.89	45.53	0.056
HDCES	57	0.995	-6.22	50.22	0.045
HCCES	57	0.996	-4.66	48.68	0.050

Ta = the ambient temperature; T_0 is the initial temperature of the body; λ = the constant rate of cooling (°C.h^{-1}); R^2 = coefficient of determination

[2]HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

Table 8: Test comparing the rates constant for deep tissue of treatment groups against that of the reference group (LCCNES) in plant B

	F – value	Std.Error	*P*-value
Intercept	0.059	0.002	<0.0001
[1]LDCNES	-0.006	0.003	0.0929
LCCES	-0.005	0.003	0.104
LDCES	-0.003	0.003	0.378
HCCNES	-0.003	0.003	0.302
HDCNES	-0.010	0.003	0.00431
HCCES	-0.009	0.003	0.00546
HDCES	-0.014	0.003	1.78

[1]HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

Table 9: Parameter estimates of the exponential cooling model fitted to surface tissue temperature in plant B

ID	n	R2	Ta	(T0-Ta)	λ
1LCCNES	57	0.993	1.83	19.85	0.14
LCCES	57	0.995	1.29	16.19	0.13
LDCES	57	0.986	2.15	21.75	0.18
LDCNES	57	0.994	1.44	20.36	0.16
HCCES	57	0.986	1.54	20.20	0.14
HCCNES	57	0.997	1.27	21.84	0.14
HDCES	57	0.997	1.94	23.03	0.14
HDCNES	57	0.993	1.69	19.61	0.14

Ta = the ambient temperature; T_0 is the initial temperature of the body; λ = the constant rate of cooling (°C.h^{-1}); R^2 = coefficient of determination

[1]HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

Table 10: Test comparing the rates constant for surface temperature of treatment groups against that of the reference group (LCCNES) in plant B

	F- value	Std.Error	*P*-value
Intercept	0.138	0.004	< 0.0001
[1]LDCNES	0.021	0.006	<0.001
LCCES	-0.003	0.006	0.626
LDCES	0.043	0.006	< 0.0001
HCCNES	0.001	0.006	0.896
HDCNES	0.003	0.006	0.610
HCCES	0.005	0.006	0.3560
HDCES	0.006	0.006	0.263

[1]HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

Table 11: Parameter estimates of the exponential cooling model fitted to deep tissue temperature in plant A

ID	n	R^2	T_a	T_0-T_a	λ
[1]LCCNES	39	0.997	1.42	40.39	0.085
LDCNES	39	0.995	-9.77	53.39	0.044
LDCES	39	0.993	-6.23	50.04	0.051
LCCES	39	0.993	-1.18	44.20	0.067
HDCNES	39	0.994	-70.57	111.99	0.016
HCCNES	39	0.991	-0.31	41.25	0.069
HCCES	39	0.992	-4.97	46.57	0.053
HDCES	39	0.995	-35.49	77.47	0.027

Ta = the ambient temperature; T_0 is the initial temperature of the body; λ = the constant rate of cooling (°C.h^{-1}); R^2 = coefficient of determination

[1]HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

Table 12: Test comparing the chilling rates for deep tissue of treatment groups against that of the reference group (LCCNES) in plant A

	F – value	Std.Error	*P*-value
Intercept	0.085	0.005	<0.0001
[1]LDCNES	-0.041	0.008	<0.0001
LDCES	-0.034	0.008	<0.0001
LCCES	-0.018	0.008	0.022
HDCNES	-0.069	0.008	<0.0001
HCCNES	-0.016	0.008	0.046
HCCES	-0.031	0.008	<0.0001
HDCES	-0.058	0.008	<0.0001

[1]HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

Table 13: Parameter estimates of the exponential cooling model fitted to surface tissue temperature in plant B

ID	n	R^2	T_a	(T_0-T_a)	λ
[1]LCCNES	57	0.994	-1.25	17.72	0.113
LDCNES	57	0.988	-2.51	20.34	0.082
LDCES	57	0.991	-1.43	18.88	0.119
LCCES	57	0.988	-1.91	16.70	0.086
HDCNES	57	0.992	-4.61	23.51	0.072
HCCNES	57	0.992	-3.40	20.06	0.072
HCCES	57	0.985	-2.67	17.25	0.076
HDCES	57	0.991	-9.86	26.66	0.041

Ta = the ambient temperature; T_0 is the initial temperature of the body; λ = the constant rate of cooling (°C.h^{-1}); R^2 = coefficient of determination

[1]HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

Table 14: Pairwise comparisons of the rates of chilling for surface temperature of treatment groups against that of the reference group (LCCNES) in plant A

	Value	Std.Error	*P*-value
Intercept	0.113	0.005	<0.0001
[1]LDCNES	-0.030	0.006	<0.0001
LDCES	0.007	0.007	0.301
LCCES	-0.026	0.007	0.0001
HCCNES	-0.041	0.006	<0.0001
HDCNES	-0.041	0.006	<0.0001
HCCES	-0.037	0.007	<0.0001
HDCES	-0.071	0.006	<0.0001

[1]HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

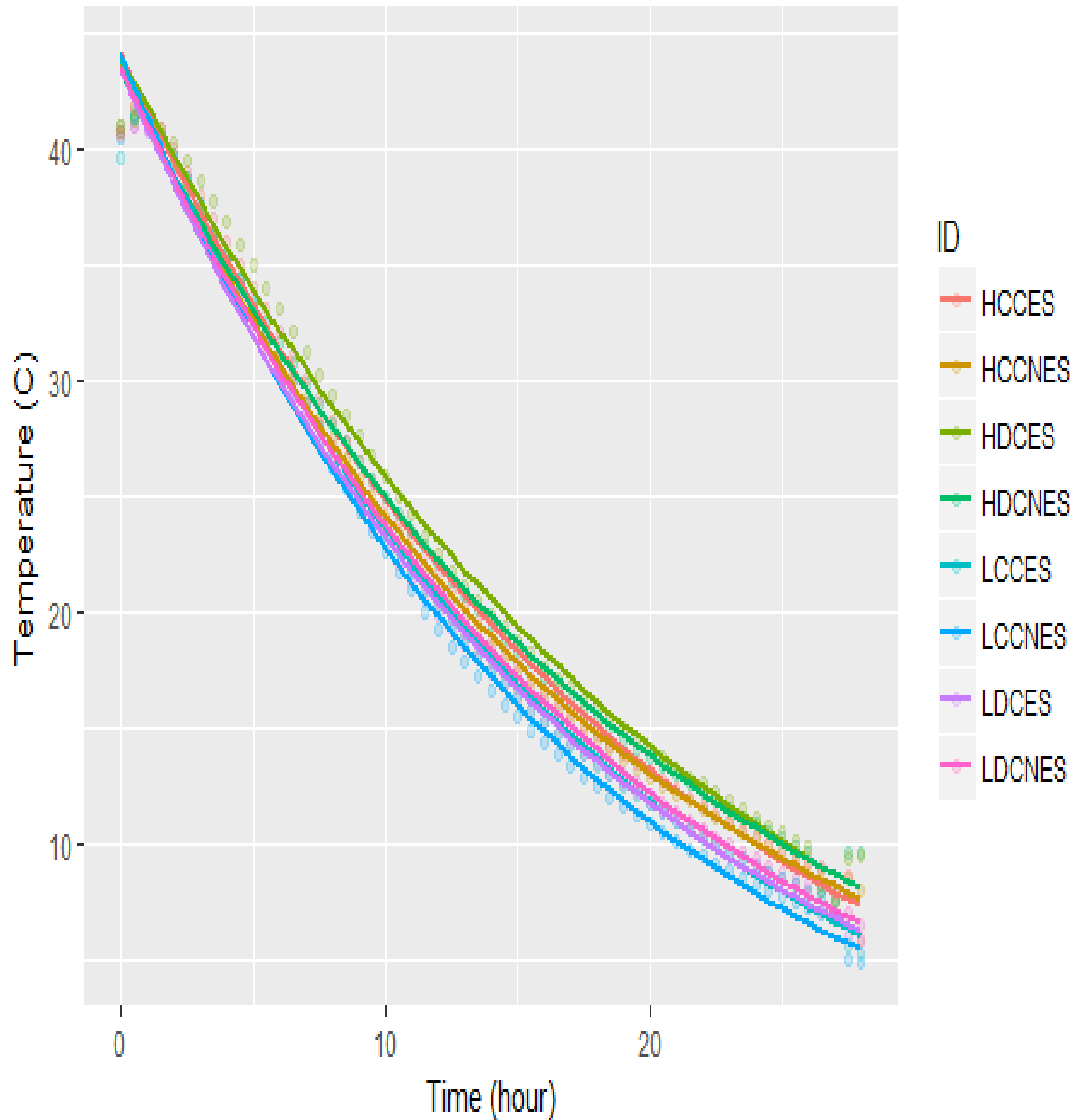

Figure 1: Fitted curves overlaid over observed deep tissue temperature decline of treatment groups in plant B

HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

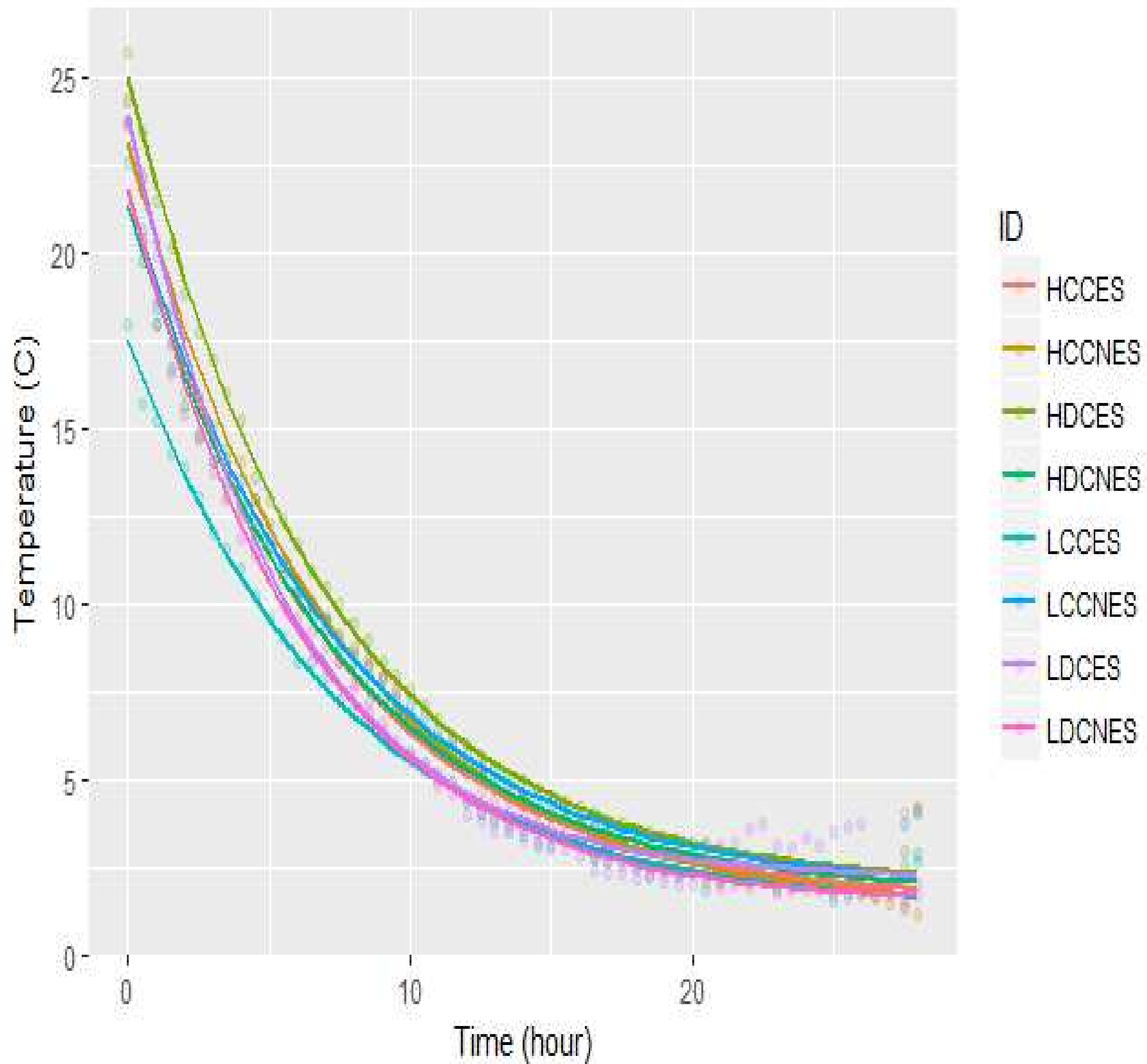

Figure 2: Fitted curves overlaid over observed surface temperature decline of treatment groups

in plant B

HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

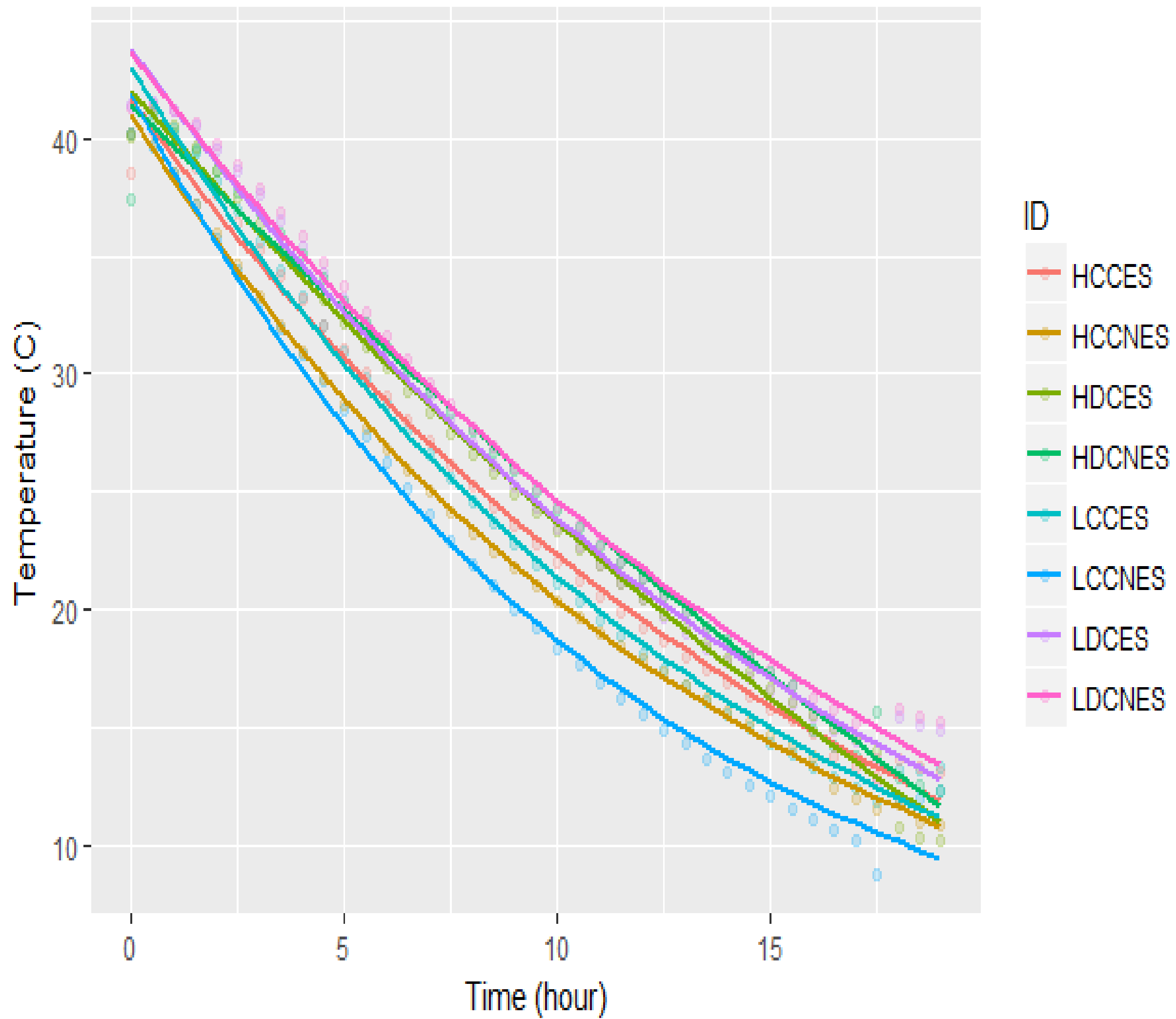

Figure 3: Fitted curves overlaid over observed deep tissue temperature decline of treatment groups in plant A

HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

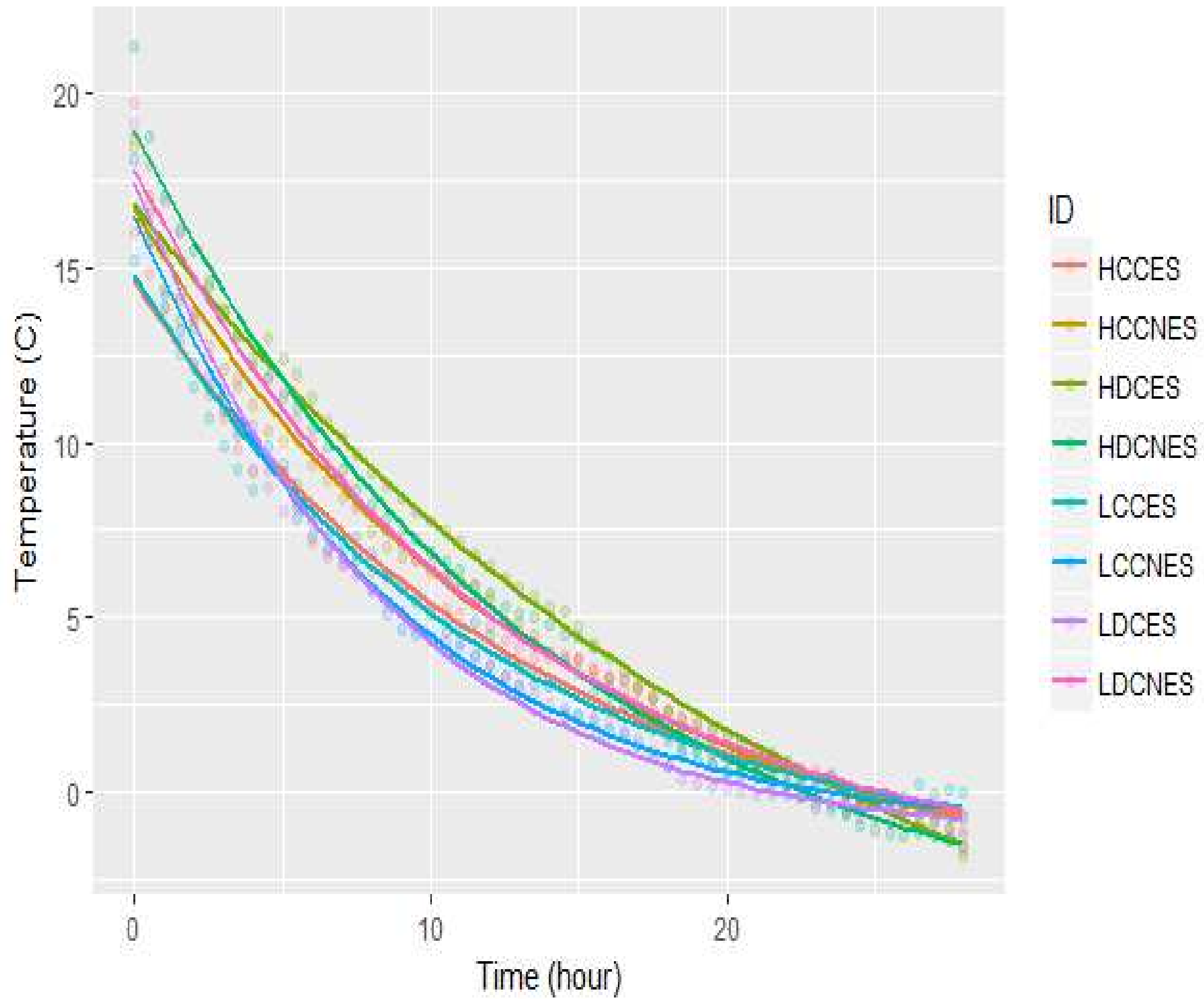

Figure 4: Fitted curves overlaid over observed temperature decline of treatment groups in plant B

HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

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CHAPTER III

UNDERSTANDING THE IMPACT OF CARCASS SIZE, RATE OF CHILLING, AND ELECTRICAL STIMULATION ON BEEF TENDERNESS

Introduction

Variability in carcass size and weight is posing challenges for beef producers for a variety of reasons including inconsistencies in product quality and eating satisfaction (NCBA, 2016). According to the recent National Beef Quality Audit (NBQA), the lowest hot carcass weight (HCW) registered in 2016 was greater than the highest HCW registered in 2010 (Boykin et al., 2017b). Previous studies indicated that carcass size has significantly increased over the years due to continual improvements in genetic selection, technologies, nutrition, health, and welfare, low cattle numbers, and demand for more beef (Dahlen et al., 2014, White et al., 2015, Sneeringer et al., 2015, Capper, 2011).

Beef producers are faced with the challenge of achieving adequate chilling rates among heavier carcasses with (Maples et al., 2018). More specifically, heavier beef carcasses challenge cooler and rail capacities (Savell et al., 2005), are harder to chill in infrastructures that were conceived for shorter and lighter carcasses (Maddock, 2015), and require substantially more time to reach acceptable deep tissue temperatures (Stopforth and Sofos, 2005). Nonetheless, the obvious economic advantages of reduced chilling times and product turnover in coolers incentivize beef packers to consider shorter chilling periods (Aalhus et al., 2001). Chilling heavier carcasses with lighter ones without prior segregation creates differences in chilling rates (Klauer et al., 2018) and postmortem metabolism that could be contributing to inconsistencies in tenderness and juiciness.

The relationship between temperature decline and postmortem metabolism is of primary interest in the management and production of a consistently high quality beef (Savell et al., 2005). Very rapid or delay chilling have been associated with an inadequate pH decline and rigor onset, which could result in issues of cold - induced shortening/toughening (Locker and Hagyard, 1963), heat ring, and/or heat conditioning (Strydom et al., 2005).

Domestic US beef retailers, purveyors, and foodservice costumers have identified inconsistencies in tenderness as one of the top five challenges (Shook et al., 2008). Beef consumers continue to consistently rank tenderness as a top palatability traits along with flavor and juiciness (Huffman et al., 1996, Platter et al., 2003, O'Quinn et al., 2018). Hence, the beef industry must continue to study the impact of postmortem processes and management practices on tenderness of current cattle as highlighted by Tatum et al. (2000) in the total quality management for beef producers. While summarizing post-harvest practices for enhancing beef tenderness, Smith et al. (2008a) indicated that a suitable combination of chilling rate and electrical stimulation could be used to achieve higher beef tenderness and quality. Savell et al. (2005) suggested that more studies be conducted to identify the best chilling protocols for heavy weight carcasses. However, it appears that a few published studies have investigated the impact of chilling and electrical stimulation in relation to variability in carcass size and weight on beef quality.

Therefore, this study sought to determine and identify the relation of carcass size, chilling, and electrical stimulation on beef tenderness to make recommendations for beef packing processors.

Material and methods

Carcass identification and selection, electrical stimulation and chilling

Carcass identification, selection, electrical stimulation and chilling were described in Djimsa et al, (2018). Briefly, cattle (N = 162, < 30 month) were randomly selected at two beef processing

plants in the US (Plant A and Plant B). The right or left side of each carcass was stimulated (ES) while the matching side remains non-stimulated (NES). In plant A, high voltage electrical stimulation was utilized while low voltage electrical stimulation was used in plant B. Matched side pairs were then chilled under conventional spray chilling (CC) or delay spray chilling (DC). At the conclusion of the chilling period, carcass data were collected including marbling score, carcass maturity (skeletal, lean, and overall), sex class (heifer, steer), hot carcass weight, fat thickness, ribeye area, and final yield grade. Following the collection of grade factors, short loins were removed from matched carcass sides for Warner-Bratzler Shear Force (WBSF) and trained sensory panel appraisal.

Aging

Short loins (Longissimus muscles, LM) were transported to the Center for Meat Safety and Quality meat laboratory at Colorado State University (Fort Collins, United States). Loins were fabricated into 4 sections of about 6.35 cm. Sections were randomly assigned to 14, 21, 28, or 35 d aging period, vacuum packaged, and aged at 0 – 2°C to the designated aging period. At the conclusion of each aging period, loin sections were frozen (-20°) and kept frozen until further analysis. Each frozen section was cut into two 2.5 cm thick steaks using a band saw. One steak was allotted to shear force and one to trained sensory analysis.

Warner-Braztler shear force (WBSF) and Slice shear force (SSF)

Previously frozen steaks designated for shear force were randomly assigned to a cooking day and thawed at 0 – 2 °F for 36 – 48 h before cooking. Thawed steaks were cooked to a peak internal temperature of 70 °C using a combination convection oven (Model SCC WE 61 E; Rational, Landsbergam Lech, Germany) set at 205 °C with a 0% relative humidity and with the fan speed on high. The internal temperature of steaks was monitored with a thermocouple (AquaTuff™ 351

Wrap&Stow^{TM} Cooper Atkins Corp., Middlefield, CT) during cooking, and steaks were removed from the oven to achieve the desired peak internal temperature. Warner-Bratzler shear force (WBSF) and slice shear force (SSF) measurements were obtained from every steak (both measures from the same steak) using procedures described by Lorenzen et al. (2010). A Slice Shear testing machine (G-R 152, G-R Electric Manufacturing LLC, Manhattan, KS) equipped with a flat, blunt-end blade (crosshead speed: 500 mm/min, load capacity: 100 kg), resulting in a single SSF measurement for each steak. The lateral portion (~1/3) of the LM steak was used for SSF measurement. The remaining portion of each steak was allowed to equilibrate to room temperature (25°C) and a maximum number of cores (1.2 cm in diameter) were removed from each steak parallel to the muscle fibers. Each core was sheared once, perpendicular to the muscle fibers, using G-R Warner-Bratzler testing machine (G-R 151, G-R Electric Manufacturing LLC, Manhattan, KS) fitted with a Warner-Bratzler shear head (crosshead speed: 200 mm/min, load cell capacity: 100 kg). Peak shear force of each core was recorded, and the resulting values were averaged to obtain a single shear force value for each steak.

Sensory analysis

Previously frozen steaks designated for trained sensory panel analysis were randomly assigned to sensory panel sessions. Sensory panel steaks were thawed and frozen with procedures identical to those described for shear force analysis. Following cooking, steaks were sized into 1 cm cubes and served warm to panelists in individual booths under incandescent red light. Six trained panelists qualified to identify differences in myofibrillar tenderness (initial, sustained and connective tissue), juiciness (initial and sustained), and palatability attributes (beef flavor identity, roasted, brown, bloody-serumy, fat-like, umami, liver-like) evaluate each sample using a 15 point scale (1 = extremely tough, dry, or non-detectable and 15 = extremely tender, extremely juicy, or

intense) for each attribute (Adhikari et al., 2011, Philip, 2011). Each steak attribute scores were averaged for statistical analysis.

Statistical analysis

To determine the effects of carcass size, carcasses were grouped into heavy and light weight categories. Carcasses with a hot carcass weight greater than the average weight in each plant were considered heavy weight; carcasses with a carcass hot weight smaller than the average weight were light. This resulted in the following: plant A, Light, n = 40, Heavy, n = 42; plant B, Light, n = 35, Heavy, n = 45.

Grading data (hot carcass weight; ribeye area; fat thickness; preliminary yield grade; adjusted preliminary yield grade; skeletal maturity; lean maturity; overall maturity; percentage kidney, pelvic, heart fat; and final yield grade) were analyzed as a paired t-test in SAS. Warner-Bratzler Shear Force (WBSF) and Slice Shear Force (SSF) were analyzed as repeated measures in a split plot design using the Mixed Procedure of SAS. Carcass served as the whole plot, and carcass side the subplot. The treatment structure was a 2 (weight category) x 2 (chill rate) x 2 (electrical stimulation) x 4 (Aging). Fixed effects included aging, carcass size (H vs. L), chilling rate (CC vs. DC), electrical stimulation (ES vs. NES), and their interactions. Trained panel data were analyzed as a 2 (weight category) x 2 (chilling rate) x 2 (electrical stimulation) factorial treatment structure in a split plot design. Animal (ID) and electrical stimulation within the carcass side (ID x ES) were included in the models as random variables. The Kenward-Roger procedure was used to adjust for denominator degrees of freedom. Least squares means were separated using the PDIFF option with an α – level ($P \leq 0.05$).

Exponential decay models

Exponential decay models were developed to create aging curves using the following packages in R: ggplot2 (Wickham, 2016), nlme (Pinheiro et al., 2018), dplyr (Wickham et al., 2017), and caret (Kuhn et al., 2016). The Self-Starting Nls Asymptotic Regression (SSasymp) function in R (Bates and Chambers, 1992) was utilized to fit self-starting nonlinear exponential decay models onto least squares means for WBSF and SSF values. These values were generated for significant main effects of aging and interactions of aging and carcass size (heavy = H; light = L), chilling rate (CC vs DC), and electrical stimulation (ES vs NES). The exponential decay models were based on the following equation:

$$WBSF = b2 + b1exp(-b0*Aging)$$

where b0 represents the rate constant of aging, b2 represents WBSF values when aging tends to infinity (point where no improvements in aging occurs), and b1 is the difference between b2 and the initial values of WBSF when aging is zero (starting point). Since data collection was similar in both plants, the same statistical analyses were performed on data collected in both plants.

Results and discussion

Population carcass characteristics

Mean values and standard deviations for carcass traits are presented in Tables 17 and 18 for plant A and B, respectively. Most of the sample population was comprised of heifers (77.5%) in plant A and steers (75%) in plant B. The sample population in plant B was more representative of the cattle population going through different processing plants throughout the US than the sample population in plant A (Boykin et al., 2017a). These authors reported 66.5% and 33.4% of the sample population were steers and heifers respectively. Ribeye area (REA), fat thickness (FT), preliminary yield grade (PYG), adjusted preliminary yield grade (APYG), skeletal maturity

(SMAT), lean maturity (LMAT), overall maturity, and final yield grade (YG) differ ($P < 0.05$) between weight category groups in both plants. Carcasses under delay chilling had greater ($P < 0.05$) FT, PYG, and APYG than the ones under conventional chilling in plant A.

Marbling and percent of kidney, pelvic, and heart (KPH) fat were not influenced ($P > 0.05$) by treatment factors. Grade parameters were not impacted ($P > 0.05$) by electrical stimulation. These results were in agreement with Hopkins et al. (2014) who reported that electrical stimulation influenced pH decline but did not impact Australian quality grade. In contrary, other studies reported significant effects of electrical stimulation on quality grade. Roeber et al. (2000) reported that electrical stimulation reduced lean maturity scores. Savell et al. (1978b) reported significant differences in lean maturity and overall maturity between stimulated and non-stimulated. Similarly to this study, Roeber et al. (2000) and Savell et al. (1978b) did not find differences in marbling scores as a result of electrical stimulation.

In plant A, the sample population was composed of 3.12% high choice, 11.45% choice, 36.45% low choice, and 48.95% select for heavy weight carcass sides and 1.28% high choice, 12.82% choice, 37.18% low choice, and 48.72% select for light weight carcasses. Fourteen percent of the sample population represented the upper 2/3 choice for both heavy and light weight sides. In plant B, the sample population averaged 37.13% upper 2/3 choice, 52.85% low choice, and 10% select in heavy weight carcass sides. Light weight carcass sides were comprised of 35.55% upper 2/3 choice, 51.11% low choice, and 13.33% select. The final YG reported in the present study was similar to that reported by Boykin et al. (2017a) but slightly higher than that reported by Thornton et al. (2017).

Sensory results

Least squares means for sensory scores are presented in tables 19 and 20 for plant A and B, respectively. The treatment factors did not ($P > 0.05$) influence sensory scores for tenderness (initial, sustained, and connective tissue tenderness), juiciness (initial and sustained), and flavor attributes (beef flavor identity, roasted, brown, bloody-serumy, fat-like, umami) in plant A and B. There was an interaction carcass size x electrical stimulation ($P < 0.05$) for liver-like for samples in plant B. Light weight stimulated carcass sides exhibited the most intense liver-like flavor ($P < 0.05$). It is surprising that electrical stimulation affects liver-live panelists scores. These findings were supported by Savell et al. (1978b) who reported no significant differences between stimulated and control sides of beef aged for 7 and 21 d except for juiciness for sensory panel scores. Similarly, despite an increased rate and extent of pH decline in response to electrical stimulation, no differences in panel ratings were found between stimulated and non-stimulated sides in a study by Hopkins et al. (2014).

Effects of treatment factors on tenderness parameters

Least squares means for tenderness attributes (WBSF and SSF) are presented in table 21 and 22 for plant A and plant B respectively. In plant A, weight category and aging time decreased ($P < 0.05$) WBSF values. Heavy weight (HW) carcasses had significantly lower WBSF values than light weight (LW) carcasses. There was a significant improvement in tenderness through to 28 days postmortem in heavy weight carcasses. Light weight carcasses did not ($P > 0.05$) show any tenderness improvement after the initial decrease in WBSF values between 14 and 21 days of age. Light weight carcasses had slice shear force (SSF) values that were numerically greater than that of HW carcasses. Improvements in SSF values for LW carcasses were gradual ($P < 0.05$) throughout the aging period. The SSF values for HW carcasses decreased to the lowest level ($P <$

0.05) by 28 days of aging. No effect of tenderness improvement due to high voltage ES was detected in plant A.

There was a significant interaction between chilling rate and aging for WBSF values in plant B. Delay chilled (DC) carcasses had greater ($P < 0.05$) WBSF at the start of the aging period (14 d) but achieved a more significant improvement in tenderness over the aging period compared to conventionally chilled (CC) carcasses. Similar results were observed for SSF values. The effect of chilling was lost by 28 d of aging ($P < 0.05$). Low voltage electrical stimulation and carcass size did not ($P > 0.05$) influence tenderness parameters in plant B. Although improvements in tenderness (WBSF and SSF) were not detected in the present study in agreement with previous studies Hopkins et al. (2014) and Kim et al. (2013), other researchers have reported significant improvements in tenderness in response to electrical stimulation (Roeber et al., 2000, Savell et al., 1978b). Electrical stimulation parameters, as well as chilling protocols and differences in carcass size, may explain the differential response observed between the present study and previous studies.

Aging curves for treatment groups

The least squares means for WBSF values of the 8 treatment groups (HDCNES, HDCES, HCCNES, HCCES, LCCNES, LCCES, LDCNES, LDCES) resulting from the interaction between carcass size (heavy =H vs light = L), chilling rate (delay chilling = DC vs conventional chilling = CC) and electrical stimulation (ES vs NES) for plant A and plant B are plotted in Fig 13 and 16 respectively. Both plants appeared to show different aging profiles for each treatment groups. The plots indicate an exponential decay behavior. Hence, exponential decay models were fitted to observed WBSF values for each treatment group (Fig 14 and 17 for plant A and plant B

respectively). The WBSF values were used as input. Parameter estimates were obtained and presented in Tables 15 and 16 for plant A and B respectively.

The goodness of fit for plant A aging data was $R^2 = 0.93$ and for plant B $R^2 = 0.79$. Therefore, the regression model used to fit aging curves appeared more appropriate for observed aging data in plant A than in plant B. The rates of tenderization characterized by b0 (instantaneous rate of change in WBSF) for the different treatment groups showed that improvements in tenderness vary among the treatment groups. The relative numerical differences between the treatment groups may need further statistical analysis to determine whether those differences are significant.

The model was less appropriate for the stimulated conventionally chilled heavy weight sides (HCCES, $R^2 = 0.80$; plant A). In plant B, the model failed to fit HCCES, non-stimulated conventionally chilled heavy weight sides (HCCNES) and stimulated conventionally chilled light sides (LCCES). These carcass sides did not show any tenderness improvements over the aging period ($P > 0.05$). Based on tenderness thresholds (tender < 3.9 kg) reported by Shackelford et al. (1991) for beef acceptability by foodservice consumers, these sides appeared to be tender at the start (14 d) of the aging period (3.17, 2.91, 2.70 for HCCES, HCCNES, and LCCES; plant B) and stayed the same throughout the aging period. Also, the LCCES group in plant A was tender at the start of the aging period. Gruber et al. (2006) reported that *Teres major* could not be fit to a nonlinear regression model because no improvements in tenderness were observed over 28 d aging period. For the scope of this discussion, these treatments groups were not included in further analysis.

Using the nonlinear models, predicted values were determined from 14 through 35 d for treatments groups for which the model was a good fit. Daily rates of change and aging response were determined and presented in Table 17 and 18 for plant A and B. The daily rates of changes

were determined by deriving the exponential decay models to the first order and computing at specific dates using the parameter estimates. The aging response was computed as a difference in predicted WBSF at 14 d and 35 d. Percentage of tenderness improvement relative to the aging response was calculated for specific days postmortem with 14 d being 0% (relative start day) and 35 d being 100%. Least squares means of WBSF for the LL of all treatment groups at 14, 21, and 28 d were lower than that reported by Gruber et al. (2006) for *Longissimus dorsi*.

In plant B, treatment groups HDCES and LCCNES showed the slowest aging response over the aging period. Based on aging response classes adopted from Gruber et al. (2006) and adapted to this study's aging period (14 through 35 d), it appeared that HDCES and LCCNES were low and moderately low and required 31 d and 30 d to complete more than 95% of tenderization respectively. Treatment groups HDCNES, LLDCES, and LDCNES required 22, 23, and 25 d postmortem to complete more than 95% tenderization. In plant A, delay chilled light weight carcass groups (LDCES and LDCNES) had the shortest aging time (28 d) compared to conventionally chilled light weight groups (LCCES and LCCNES, 31 d).

In the heavy weight group, stimulated delay-chilled carcass sides showed the shortest tenderization time (28 d). Surprisingly, the HCCES required the longest tenderization period (33 d). Based on aging response classes reported by (Gruber et al., 2006) it would seem that the aging response for treatments groups in this study were moderately low to low. This is more likely because most of the tenderness improvement may have occurred before to 14 d. In support, Gruber et al. (2006) reported that 65.8% of aging occurred by 14 d postmortem in LD samples.

Electrical stimulation is one of the postharvest practices to improve postmortem tenderness and has been extensively investigated. Several studies have reported positive effects of ES on tenderness (Savell et al., 1979, Roeber et al., 2000, Davey et al., 1976a, Hwang and Thompson,

2001, Eikelenboom et al., 1985). Although the results of the current study are not in agreement with some of the previous research, it indicates that electrical stimulation exerts certain effects on postmortem metabolism and beef quality. In support, some investigations had reported no effect (Kim et al., 2013, Hopkins et al., 2014) or adverse effects of electrical stimulation (Marsh et al., 1981) on tenderness ratings as well as WBSF values of beef. This suggests that the efficacy of electrical stimulation is very much dependent upon a variety of postmortem management practices as well as intrinsic characteristics of the muscle (fiber type).

In the present study, carcass size and aging happened to have greater influence on tenderness and tenderization than electrical stimulation. This is in agreement with the results of Juárez et al. (2012) and Juárez et al. (2016) who reported that the relative contribution of electrical stimulation to tenderness improvement was lower compared to that of aging period. These authors reported that ES explained only 12% of the variability in tenderness compared to 45% due to aging. Furthermore, Juárez et al. (2016) also suggested that the increased carcass size might be contributing to inconsistencies in tenderness as a result of differences in chilling rates.

As carcass size and weight trend upward for beef in in the USA, beef processors are concerned about the efficacy of postmortem management processes (including ES) to improve beef quality and minimize product - to - product variability. In chapter II, we reported that electrical stimulation interacted with carcass size, chilling rate, and time postmortem in influencing pH decline in the *longissimus lumborum* (LL). The study showed that light weight carcass sides under conventional chilling had the most significant response to electrical stimulation as indicated by the pH decline. Stimulated sides had a more rapid and extensive pH decline than non-stimulated sides.

In agreement with Klauer et al. (2018), the results in chapter II also showed that heavy carcasses had slower chilling rates. Furthermore, these authors reported that heavy weight

carcasses had a faster rate of pH decline subsequent to a slower heat removal rate. Kuffi et al. (2018) also reported that rapid pH decline is a consequence of slow chilling rates. It is unlikely that heavy weight carcasses are susceptible to cold shortening under conventional chilling protocols because of the slower chilling rates.

Cold shortening becomes an issue when muscle temperatures fall below 10 - 15°C simultaneously with the muscle pH at around 6. Such conditions are unlikely under current chilling practices and less so for heavy weight carcasses. Improvements in tenderness reported herein may be the result of multifaceted effects integrating carcass size, chilling rates, biological variability, electrical stimulation, and other unforeseen factors. For instance, in plant B for a short period (20 to 25 min) following electrical stimulation, carcasses were exposed to hot (38 - 40°C) environmental temperatures (data not presented) as they traveled through to blast chillers.

Given proteolysis has been shown to start right at slaughter (Hwang et al., 2003), high temperatures early postmortem may result in increased rate of tenderization. Marsh et al. (1981) reported appreciable improvements in tenderness by manipulating temperature decline in carcasses by exposing them to high environmental temperatures (37°C) or by heavy fat cover early postmortem. It was also suggested by Rhee and Kim (2001) that high temperatures (30°C) were an effective way to alter glycolytic rate early postmortem and impact tenderness and tenderization of beef. All treatment groups in plant B resulted in steaks that were tender (< 3.9 kg) based on tenderness thresholds reported by Shackelford et al. (1991).

It has been proposed that postmortem aging follows an exponential decay model. Experimental data and investigations by some researchers have provided reasonably appropriate estimates of tenderness and the tenderization process for a variety of beef muscles (Dixon et al., 2012, Gruber et al., 2006). The present study provided further evidence that aging behavior could be modeled

using an exponential decay model. Similar to previous studies, the models developed herein were appropriate fits for most of the observed data. However, the model failed to fit a few treatments groups that demonstrated no improvements in tenderness or had a behavior that did not follow a typical exponential decay model. Similar observations were made by Gruber et al. (2006) on some muscles that did not show any tenderness improvements over the chilling period.

Discrepancies between the observed and predicted WBSF depicted in this study could be attributed to many factors. Tenderness improvements have been shown to occur for the most part within 72 hours postmortem. More specifically, it has been shown that in the LL most of tenderness improvement had occurred by 14 d of conditioning (Gruber et al., 2006, Koohmaraie et al., 1988c, Lorenzen et al., 1998). This means that the present observed data and fitted range only captured the tail portion of the aging process. The longest time response to aging in the present study was 33 d postmortem. Agreeing with the results of the present study, Stolowski et al. (2006) reported that the LD was tender but had a slow response to aging with improvements detected up to 42 d postmortem.

Conclusion

The upward trend of carcass size in the US could be contributing to tenderness inconsistencies in beef as carcasses of varying sizes are being processed under the same conditions. Under the conditions provided in this study, the chilling of carcasses of varying sizes resulted in differential chilling rates and tenderness. Heavy weight carcasses performed significantly better in terms of tenderness and tenderization as a result of a slower chilling rate coupled with a faster pH decline. Exponential decay model predictions, in agreement with observed data, showed that the LL muscle is likely to improve in tenderness up to 33 days postmortem even though most of the tenderization is completed by 14 d. Delay chilling, and increased carcass size appeared to improve sensory

tenderness scores as well as objective tenderness. The combination of carcass size, method of chilling, and electrical stimulation has more proven more useful in improving tenderness profile of light weight carcasses than heavy weight carcasses.

Table 15: Descriptive statistics for grading data in plant A

	Heavy weight				Light Weight			
	DC[1]		CC		DC		CC	
	NES[2]	ES	NES	ES	NES	ES	NES	ES
n	20	20	22	22	20	20	20	20
[3]HCW (Kg)	447.46 ± 29.41	447.4 6± 29.41	445.2 ± 19	445.2 ± 19	362.49 ± 31.24	362 ± 31.24	360.01 ± 36.69	360.01 ± 36.69
REA	16.08 ± 2.33	16 ± 2.02	16.64 ± 1.52	16.53 ± 1.46	13.98 ± 1.97	14.4 ± 1.92	14.95 ± 1.9	14.67 ± 1.93
FT (mm)	18.38 ± 8.37	18.5 ± 8.74	14.9 ± 5.54	15.88 ± 6.43	13.46 ± 8.6	14.1 ± 9.73	11.02 ± 4.29	11.28 ± 4.67
PYG	3.81 ± 082	3.82 ± 0.86	3.47 ± 0.55	3.56 ± 0.63	3.33 ± 0.85	3.39 ± 0.96	3.09 ± 0.42	3.11 ± 046
adjPYG	3.86 ± 0.84	3.9 ± 0.84	3.6 ± 0.53	3.63 ± 0.69	3.34 ± 0.95	3.41 ± 1.05	3.13 ± 0.48	3.13 ± 0.53
Marbling	407.39 ± 73.37	400 ± 75.59	426.3 ± 84.74	432.1 ± 88.41	429.5 ± 83.76	429 ± 79.03	385 ± 55.39	398.5 ± 60.55
SMat	69.57 ± 51.39	75.2 ± 47.08	53.33 ± 29.44	49.58 ± 29.26	49 ± 35.08	66.5 ± 63.02	76 ± 88.46	71.5 ± 90.51
LMat	107.39 ± 126.68	106 ± 122.1	82.5 ± 104.3	77.08 ± 93.78	43 ± 41.18	45 ± 43.59	26 ± 24.58	30 ± 28.84
OMat	84.13 ± 64.11	86.7 ± 59.57	69.17 ± 59.14	64.17 ± 58.55	48.25 ± 31.84	63 ± 60.18	53 ± 51.67	50 ± 50.86
%KPH	3.74 ± 0.47	3.91 ± 0.39	4.19 ± 1.06	3.85 ± 0.52	3.55 ± 0.92	3.58 ± 0.89	3.7 ± 0.77	3.58 ± 0.73
YG	3.66 ± 1.61	3.72 ± 1.49	3.21 ± 0.88	3.27 ± 0.95	3.1 ± 1.38	3.05 ± 1.48	2.56 ± 0.84	2.65 ± 0.85

[1]DC = Delayed spray-chilling; CC = conventional spray-chilling;
[2]NES = Non-stimulated; ES = stimulated
[3]HCW = Hot carcass weight; REA = ribeye area; FT= fat thickness; PYG = preliminary yield grade; adjPYG = adjusted preliminary yield grade; SMat = skeletal maturity; LMat = lean maturity; OMat = overall maturity; %KPH = percentage kidney, pelvic, heart fat; YG = yield grade.

Table 16:Descriptive statistics for grade data in plant B

	Heavy weight				Light Weight			
	DC[1]		CC		DC		CC	
	NES[2]	ES	NES	ES	NES	ES	NES	ES
n	25	25	20	20	16	16	19	19
[3]HCW (Kg)	418.07 ± 27.15	418.07 ± 27.15	420.55 ± 25.59	420.55 ± 25.59	357.15 ± 23.32	357.15 ± 23.32	353.8 ± 24.24	353.8 ± 24.24
REA	15.29 ± 1.55	15.12 ± 1.14	14.28 ± 0.79	14.49 ± 1.1	13.54 ± 1.31	13.68 ± 1.5	13.53 ± 0.93	13.44 ± 1.29
FT (mm)	18.38 ± 3.52	14.94 ± 3.72	17.54 ± 4.05	16.67 ± 5.99	14.34 ± 4.69	13.56 ± 4.73	13.42 ± 4.19	13.7 ± 4.38
PYG	3.75 ± 0.35	3.46 ± 0.37	3.73 ± 0.4	3.64 ± 0.59	3.4 ± 0.46	3.33 ± 0.47	3.32 ± 0.42	3.35 ± 0.43
adjPYG	3.67 ± 0.38	3.45 ± 0.4	3.86 ± 0.53	3.73 ± 0.56	3.44 ± 0.46	3.42 ± 0.42	3.36 ± 0.55	3.43 ± 0.48
Marbling	462.5 ± 70.29	475.6 ± 77.28	492.6 ± 98.99	505.3 ± 95.76	484.23 ± 92.31	475.4 ± 81.1	484.21 ± 85.78	483.16 ± 89.2
SMat	41.25 ± 37.57	43.75 ± 40.48	50.53 ± 43.01	54.21 ± 50.7	33.85 ± 25.93	34.23 ± 25.8	24.21 ± 18.95	26.32 ± 22.41
LMat	46.25 ± 27.29	45 ± 28.05	52.63 ± 30.7	50.53 ± 31.18	43.85 ± 20.41	45 ± 17.72	42.11 ± 17.51	41.05 ± 15.6
OMat	43.75 ± 31.49	44.38 ± 32.09	51.58 ± 34.24	52.37 ± 36.64	38.85 ± 21.04	40 ± 21.07	32.63 ± 12.18	33.68 ± 16.32
%KPH	3.09 ± 1.76	3.45 ± 1.44	4.18 ± 1.54	3.92 ± 1.53	3.68 ± 1.65	3.96 ± 1.65	3.74 ± 1.16	3.92 ± 1.52
YG	3.66 ± 1.24	2.76 ± 1	3.98 ± 0.76	3.77 ± 0.71	3.22 ± 0.84	3.16 ± 0.86	3.2 ± 0.69	3.29 ± 0.7

[1]DC = Delayed spray-chilling; CC = conventional spray-chilling;
[2]NES = Non-stimulated; ES = stimulated
[3]HCW = Hot carcass weight; REA = ribeye area; FT= fat thickness; PYG = preliminary yield grade; adjPYG = adjusted preliminary yield grade; SMat = skeletal maturity; LMat = lean maturity; OMat = overall maturity; %KPH = percentage kidney, pelvic, heart fat; YG = yield grade.

Table 17: Effects of carcass size (H vs L), electrical stimulation (ES vs NES), and chilling rate (CC vs DC) on sensory panel ratings in plant A

Variable			Heavy weight		Light weight		DC	CC	ES	NES
Sensory attributes (n=)	Initial tenderness		9.31		9.4		9.53	9.19	9.38	9.33
		SEM			0.2			0.2		0.16
	Sustained tenderness		9.25		9.32		9.42	9.16	9.31	9.21
		SEM			0.2			0.21		0.17
	Connective tissue amount		9.19		9.16		9.22	9.17	9.38	8.97
		SEM			0.2			0.16		0.2
	Initial juiciness		6.62		6.68		6.74	6.56	6.6	6.7
		SEM			0.13			0.12		0.11
	Sustained juiciness		6.2		6.31		6.29	6.21	6.27	6.24
		SEM			0.13			0.11		0.1
	Beef flavor identity		7.67		7.61		7.6	7.68	7.61	7.67
		SEM			0.2			0.16		0.2
	Brown		5.55		5.28		5.38	5.41	5.4	5.41
		SEM						0.11		0.1
	Roasted		5.41		5.41		5.45	5.37	5.38	5.44
		SEM			0.1			0.1		0.08
	Bloody/Serumy		1.02		1.16		1.09	1.1	1.04	1.15
		SEM			0.05			0.05		0.06
	Metallic		1.8		1.82		1.8	1.82	1.76	1.86
		SEM			0.05			0.04		0.05
	Umami		1.48		1.43		1.46	1.45	1.47	1.43
		SEM			0.06			0.06		0.07
	Liver-like*		DC	CC	DC	CC			0.19	0.23
			0.16ax	0.16ax	0.12ax	0.40by				0.04
		SEM	0.04		0.04					
	Fat-like		1.07		1.01		1.05	1.04	1.09	0.99
		SEM			0.05			0.04		0.05

Liver-like* = interaction between carcass size and chilling rate; [1]DC = Delayed spray chilled; CC = conventional spray chilled, [2]ES = electrical stimulated; NES = Non-stimulated

[1]DC = Delayed spray chilled; CC = conventional spray chilled, [2]ES = electrical stimulated; NES = Non-stimulated

Table 18: Effects of carcass size (H vs L), electrical stimulation (ES vs NES), and chilling rate (CC vs DC) on sensory panel ratings in plant B

Variable			Heavy weight	Light weight	DC	CC	ES	NES
Sensory attributes (n=)	Initial tenderness		9.8	10.08	9.81	10.08	9.94	9.95
		SEM		0.26		0.27		0.16
	Sustained tenderness		9.86	10.09	9.86	10.09	9.97	9.99
		SEM		0.33		0.33		0.16
	Connective tissue amount		7.01	7.09	6.97	7.13	7.03	7.07
		SEM		0.15		0.15		0.12
	Initial juiciness		6.69	6.7	6.58	6.81	6.62	6.78
		SEM		0.14		0.14		0.12
	Sustained juiciness		7.54	7.52	7.57	7.49	7.51	7.55
		SEM		0.15		0.15		0.12
	Beef flavor identity		5.71	5.87	5.82	5.75	5.76	5.82
		SEM		0.13		0.13		0.11
	Brown		5.21	5.61	5.38	5.44	5.34	5.48
		SEM		0.14		0.14		0.12
	Roasted		1.19	1.38	1.35	1.23	1.35	1.23
		SEM		0.07		0.06		0.07
	Bloody/Serumy		1.87	1.85	1.87	1.86	1.87	1.85
		SEM		0.07		0.06		0.07
	Metallic		1.2	1.17	1.11	1.26	1.21	1.16
		SEM		0.08		0.08		0.06
	Umami		1.63	1.61	1.67	1.57	1.6	1.64
		SEM		0.08		0.08		0.06
	Liver-like		0.23	0.19	0.19	0.23	0.2	0.21
		SEM		0.03		0.03		0.03
	Fat-like		0.3	0.2	0.22	0.29	0.22	0.28
		SEM		0.04		0.04		0.04

[1]DC = Delayed spray chilled; CC = conventional spray chilled, [2]ES = electrical stimulated; NES = Non-stimulated

Table 19: Effects of carcass size (H vs L), electrical stimulation (ES vs NES), and chilling rate (CC vs DC) on WBSF and SSF values in plant A

		Heavy weight	Light weight	DC[1]	CC	ES[2]	NES
WBSF (Kg)	14 d	4.04c[3]x[4]	4.35bx	4.19cx	4.19cx	4.12cx	4.27cx
	21 d	3.39bx	3.48ax	3.38bx	3.49bx	3.48bx	3.4bx
	28 d	2.85ax	3.26ay	3.08ax	3.03ax	3.06ax	3.05ax
	35 d	3.04ax	3.19ax	3.19ax	3.04ax	3.09ax	3.13ax
SEM			0.2		0.16		0.8
P -value			0.022		0.71		0.77
SSF	14 d	15.20bx	16.74dy	16.68dx	15.79bx	16.34cx	16.14cx
	21 d	15.49by	14.65cx	15.06cx	15.08bx	15.16cx	14.98cx
	28 d	12.73ax	13.03bx	12.89bx	12.87ax	13.01bx	12.75bx
	35 d	11.48ay	10.2ax	10.22ax	11.47ax	11.24ax	10.45ax
SEM			0.47		0.47		0.7
P -value			0.03		0.54		0.15

[1]DC = Delayed spray chilled; CC = conventional spray chilled
[2]ES = electrical stimulated; NES = Non-stimulated
[3]Least squares means within a column with different letters (a-d) are different ($P < 0.05$)
[4]Least squares means within a same row for the same variable with different letters (x-y) are different ($P < 0.05$)

Table 20: Effects of carcass size (H vs L), electrical stimulation (ES vs NES), and chilling rate (CC vs DC) on WBSF and SSF values in plant B

		Heavy weight	Light weight	DC[1]	CC	ES[2]	NES
WBSF	14 d	3.33b[3]x[4]	3.07by	3.40cy	2.98bx	3.09bx	3.31bx
	21 d	2.65ax	2.62ax	2.72bx	2.54ax	2.66ax	2.61ax
	28 d	2.55ax	2.49ax	2.48ax	2.55ax	2.53ax	2.51ax
	35 d	2.58ax	2.51ax	2.62ax	2.58ax	2.54ax	2.66x
SEM			0.12		0.12		0.12
P -value			0.04		0.032		0.37
SSF	14 d	13.77cx	13.08bx	13.8cx	13.05bx	13.50bx	13.34bx
	21 d	9.76ax	10.04ax	9.58ax	10.21ax	10.01ax	9.78ax
	28 d	10.09bx	10.06ax	10.68bx	9.88ax	10.49ax	10.06ax
	35 d	9.21ax	10.17ax	8.99ax	10.39ay	9.55ax	9.84ax
SEM			0.15		0.7		0.7
P -value			0.7		0.011		0.64

[1]DC = Delayed spray chilled; CC = conventional spray chilled

[2]ES = electrical stimulated; NES = Non-stimulated

[3]Least squares means within a column with different letters (a-c) are different ($P < 0.05$)

[4]Least squares means within a same row for the same variable with different letters (x-y) are different ($P < 0.05$)

Table 21: Parameter estimates for exponential decay model fitted to least squares means of WBSF of treatment groups for plant A

	Parameter estimates[1]			
Treatment groups	R^2	b2	b1	b0
[2]HCCES	0.8	2.55	4.14	0.07
HCCNES	0.98	2.92	14.54	0.18
HDCES	0.99	3.09	20.92	0.22
HDCNES	0.9	2.84	10.65	0.15
LCCES	0.97	2.91	9.48	0.13
LCCNES	0.99	2.95	11.48	0.15
LDCES	0.98	3.07	16.86	0.20
LDCNES	0.99	3.34	18.48	0.20

[1]Parameter estimates were determined using the nonlinear regression model: $WBSF = b2 + b1 \exp(-b0t)$

[2]HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

Table 22: Parameter estimates for exponential decay model fitted to least squares means of WBSF of treatment groups for plant B

Treatment groups	Parameter estimates[1]			
	R^2	b2	b1	b0
[2]HDCES	0.92	2.57	7.18	0.13
HDCNES	0.97	2.66	270.49	0.41
LCCNES	0.96	2.54	6.62	0.17
LDCES	0.98	2.45	60.15	0.33
LDCNES	0.99	2.42	58.78	0.29

Parameter estimates were determined using the nonlinear regression model: WBSF = b2 + b1 exp(−b0t)

[2]HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

Table 23: Rate of change in Warner-Bratzler shear force (WBSF; kg/instantaneous unit of time) for treatment groups in plant A as estimated by nonlinear regression models1 fitted to least squares means of treatment groups

	Days postmortem (kg, WBSF/d)			
Treatment groups	14	21	28	35
[2]HCCES	-0.10	-0.06	-0.04	-0.024
HCCNES	-0.20	-0.06	-0.01	-0.004
HDCES	-0.21	-0.04	-0.01	-0.002
HDCNES	-0.19	-0.07	-0.02	-0.008
LCCES	-0.19	-0.08	-0.03	-0.012
LCCNES	-0.21	-0.07	-0.02	-0.009
LDCES	-0.20	-0.05	-0.01	-0.003
LDCNES	-0.22	-0.05	-0.01	-0.003

[1]Nonlinear regression model: WBSF = b2 + b1 exp(−b0t). Rate of change calculated by first derivative: dWBSF/dt = −b0b1 exp(−b0t)

[2]HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

Table 24: Rate of change in Warner-Bratzler shear force (WBSF; kg/instantaneous unit of time) for treatment groups in plant B as estimated by nonlinear regression models1 fitted to least squares means of treatment groups

Treatment groups	Days postmortem (kg, WBSF/d)			
	14	21	28	35
[2]HDCES	-0.15	-0.06	-0.022	-0.009
HDCNES	-0.36	-0.02	-0.001	-0.0001
LCCNES	-0.10	-0.03	-0.009	-0.003
LDCES	-0.20	-0.02	-0.002	-0.0002
LDCNES	-0.28	-0.03	-0.004	-0.0006

[1]Nonlinear regression model: WBSF = b2 + b1 exp(−b0t). Rate of change calculated by first derivative: dWBSF/dt = −b0b1 exp(−b0t)

[2]HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

Table 25: Warner-Braztler shear force (WBSF) for treatment groups at 14 d, aging response (change in shear force through 35 d), percentage of that change completed at each postmortem aging period, response class, and day 95% of the aging response was completed in plant A

	Time postmortem (d)						
	14 d WBSF[1]	Aging Response[4], kg	21	28	35	Response class[2]	95% day[3]
[5]HCCES	4.05	1.17	51.28	82.05	100	Moderate	33
HCCNES	4.04	1.09	74.31	94.45	100	Moderate	29
HDCES	4.06	0.96	79.17	96.87	100	Moderately low	28
HDCNES	4.12	1.23	68.29	91.87	100	Moderate	30
LCCES	4.39	1.39	64.03	89.93	100	Moderate	31
LCCNES	4.38	1.37	67.88	91.97	100	Moderate	31
LDCES	4.07	0.98	76.53	97.92	100	Moderately low	28
LDCNES	4.46	1.1	76.36	95.45	100	Moderate	28

[1]Predicted WBSF at 14 d

[2]Response class = type of aging response

[3]95% day = aging time required to complete 95% of tenderization

[4]Aging response = predicted WBSF at 14 d minus predicted WBSF value at 35 d

[5]HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

Table 26: Warner-Braztler shear force (WBSF) for treatment groups at 14 d, aging response (change in shear force through 35 d), percentage of that change completed at each postmortem aging period, response class, and day 95% of the aging response was completed in plant B

	Time postmortem (d)						
	14 d WBSF[1]	Aging Response[4], kg	21	28	35	Response class[2]	95% day[3]
[5]HDCES	3.66	1.03	65.05	90.3	100	Moderately low	31
HDCNES	3.54	0.88	94.31	100	100	Moderately low	22
LCCNES	3.06	0.61	70.41	94.83	100	Low	30
LDCES	3.14	0.58	90.16	98.36	100	Low	23
LDCNES	3.36	0.94	87.23	97.87	100	Moderately low	25
HDCES	3.66	1.03	65.05	90.3	100	Moderately low	31

[1]Predicted WBSF at 14 d

[2]Response class = type of aging response

[3]95% day = aging time required to complete 95% of tenderization

[4]Aging response = predicted WBSF at 14 d minus predicted WBSF value at 35 d

[5]HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

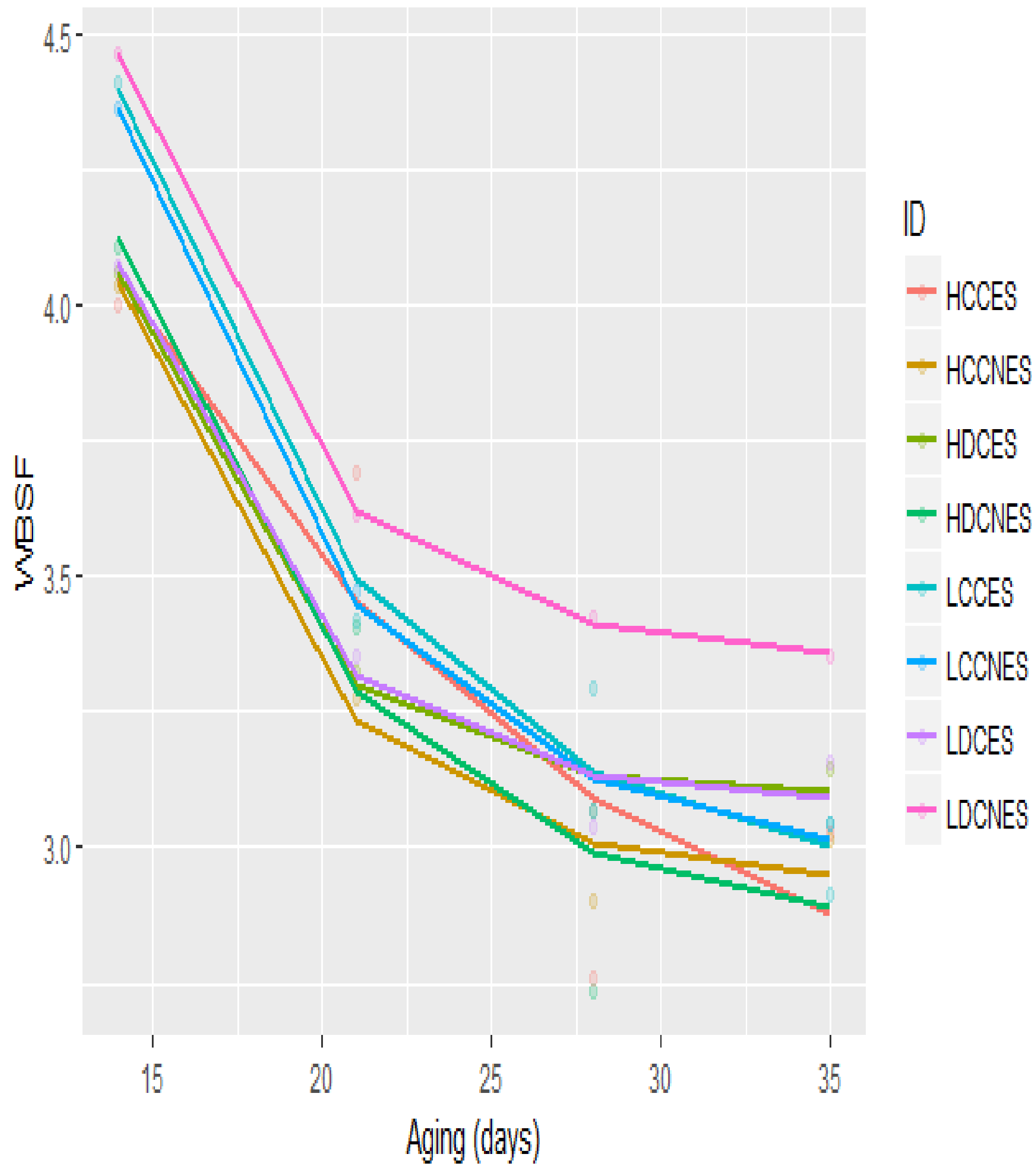

Figure 5: Predicted Aging curve vs actual WBSF in plant A

HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

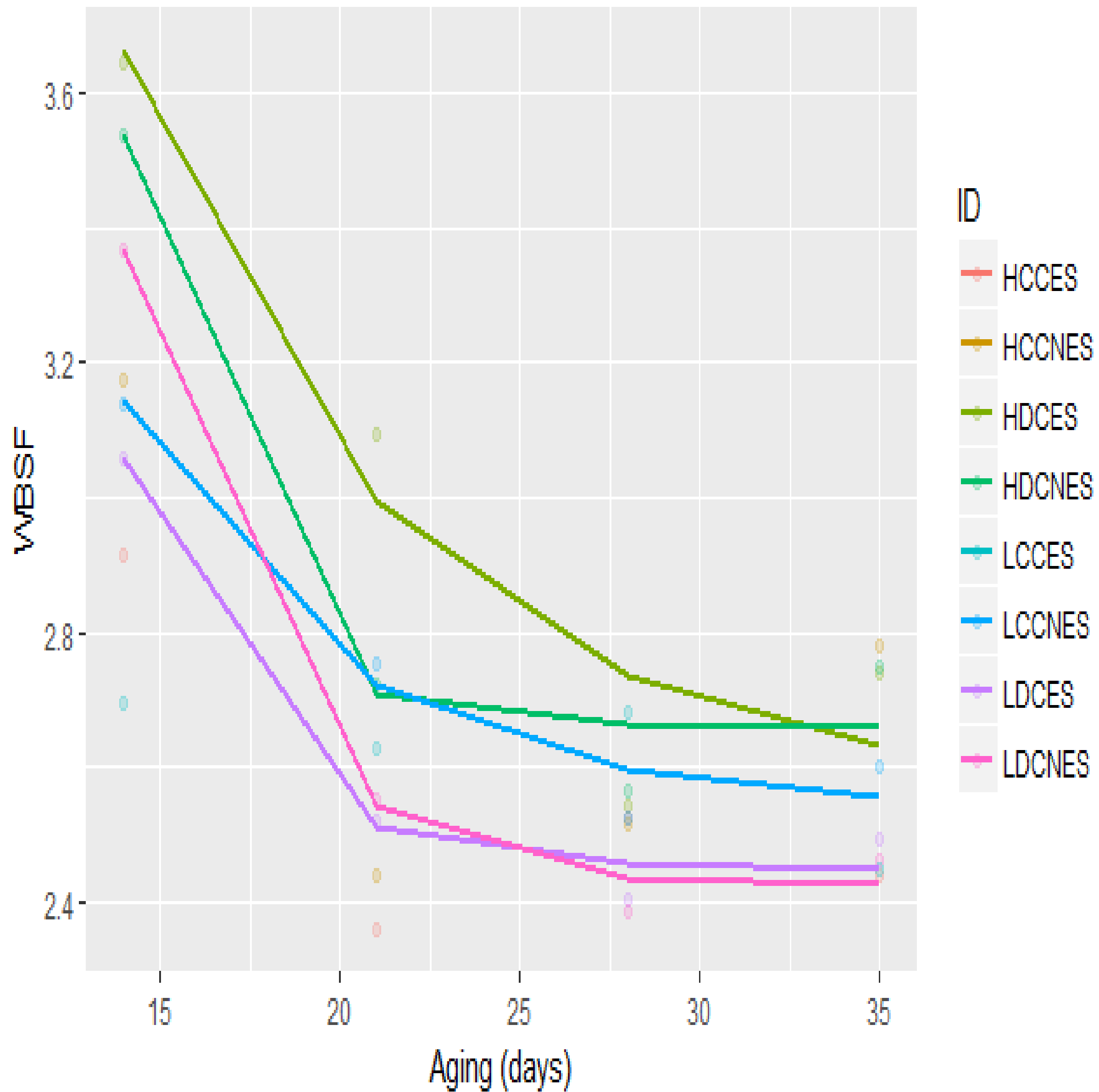

Figure 6: Predicted aging curve vs actual WBSF in plant B

HCCES = heavy weight conventionally spray-chilled carcass sides; HCCNES = heavy weight conventionally spray-chilled non-stimulated sides; HDCES = heavy weight delayed spray-chilled stimulated sides; HDCNES = heavy weight delayed spray-chilled non-stimulated sides; LCCES = light weight conventionally spray-chilled carcass sides stimulated sides; LCCNES = light weight conventionally-chilled non-stimulated sides; LDCES = light weight delayed spray-chilled stimulated sides; LDCNES = light weight delayed spray-chilled non-stimulated sides

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APPENDICES

Table 27: Effects of carcass size, chilling rate, and electrical stimulation on temperature in the Longissimus lumborum (LL), Psoas major (PM), and Semimembranosus (SM) in plant A

	Heavy weight				Light weight			
	CC[1]		DC		CC		DC	
	NES[2]	ES	NES	ES	NES	ES	NES	ES
Semimembranosus (SM)								
Time1*	36.79dx	36.72dx	37.15dx	37.38dx	39.68dy	39.39dy	39.81dy	39.56dy
Time2*	22.96cx	23.97cxy	25.08cy	23.84cxy	23.28cxy	22.66cx	23.08cx	22.32cx
Time3*	14.19bwxy	14.94bxyz	16.15bz	15.3byz	13.09bvwx	12.66bvw	12.92bwxy	11.6bv
Time4*	3.15avwy	3.81ay	3.43awy	3.44awy	2.79avwy	2.23avwy	1.55avw	1.37av
n	22	22	20	20	20	20	20	20
SEM	1.01							
P-value	0.76							
Longissimus lumborum (LL)								
Time1*	36.59dw	36.58dw	36.1dw	35.63dw	39.89dx	39.6dx	39.23dx	39.42dx
Time2*	21.7cwxy	21.08cwx	21.68cwxy	20.34cw	24.05cz	23.23cyz	22.7cxyz	23.61cyz
Time3*	12.1bw	11.54bw	12.99bw	12.1bw	13.02bw	13.22bw	13.54bw	13.13bw
Time4*	2.19aw	1.92aw	1.9aw	1.47aw	3.45aw	3.07aw	2.78aw	2.28aw
n	22	22	20	20	20	20	20	20
SEM	1.12							
P-value	0.4							
Psoas major (PM)								
Time1*	32.07x	31.72x	33.33x	33.46x	37.97y	37.64y	37.67y	37.58y
Time2*	17.33xy	16.37x	17.86xy	17.43xy	18.5xy	18.94y	19.1y	17.73xy
Time3*	8.84bxyz	8.55bxy	9.67bxyz	9.79bxyz	10.82bz	9.93byz	8.42bxy	7.77bx
Time4*	0.63ax	0.7ax	0.65ax	0.82ax	1.39ax	0.88ax	0.05ax	0.34ax
n	22	22	20	20	20	20	20	20
SEM	1.13							
P-value	0.6							

[1] CC = conventional spray chilling, DC = delay spray chilling
[2]ES = electrically stimulated; NES = non-stimulated
Time1* = initial temperature at arrival in blast chillers; Time2 = 6 h of chilling; Time3 = 12 h of chilling; Time4 = final hour of chilling
[a-d] Least squares means within a column with different letters are different ($P < 0.05$)
[v-z] Least squares means within a same row for the same variable and muscle with different letters are different ($P < 0.05$)

Table 28: Effects of carcass size, chilling rate, and electrical stimulation on pH in the Longissimus lumborum (LL), Psoas major (PM), and Semimembranosus (SM) in plant A

	Heavy weight				Light weight			
	CC[1]		DC		CC		DC	
	NES[2]	ES	NES	ES	NES	ES	NES	ES
Semimembranosus (SM)								
Time1*	6.42dy	6.26dwx	6.23cx	6.21dw	6.36cxy	y6.41d	6.18cw	6.37dxy
Time2*	5.92cyz	5.97cz	5.81bwxy	5.78cwx	5.76bwx	5.87cxyz	5.72bw	5.89cxyz
Time3*	5.75bx	5.66bwx	5.57aw	5.62bwx	5.7bx	5.68bwx	5.7bx	5.62bwx
Time4*	5.49aw	5.5aw	5.46aw	5.48aw	5.47aw	5.46aw	5.48aw	5.5aw
n	22	22	20	20	20	20	20	20
SEM	0.07							
P- value	0.6							
Longissimus lumborum (LL)								
Time1*	6.22cyz	6.26cz	6.1cxy	6.17cxyz	6.21cyz	6.19dyz	6.15cxyz	6.05cx
Time2*	5.64bxyz	5.61bxy	5.59bx	5.62bxy	5.64bxyz	5.74cxy	5.74bz	5.73byz
Time3*	5.52ax	5.52abx	5.56bx	5.58bx	5.58bx	5.61bxy	5.73by	5.58ax
Time4*	5.45ax	5.44ax	5.44ax	5.43ax	5.45ax	5.49ax	5.5ax	5.48ax
n	22	22	20	20	20	20	20	20
SEM	0.05							
P- value	0.21							
Psoas major (PM)								
Time1*	5.71bxy	5.88bz	5.71bxy	5.77bxy	5.81cyz	5.75bxy	5.65ax	5.69axy
Time2*	5.62ax	5.63ax	5.66abx	5.67ax	5.73bcx	5.65ax	5.61ax	5.63ax
Time3*	5.64abx	5.62ax	5.64abx	5.69ax	5.66abx	5.66ax	5.63ax	5.63ax
Time4*	5.62ax	5.63ax	5.62ax	5.66ax	5.63ax	5.63ax	5.6ax	5.6ax
n	22	22	20	20	20	20	20	20
SEM	0.07							
P- value	0.33							

[1] CC = conventional spray chilling, DC = delay spray chilling
[2]ES = electrically stimulated; NES = non-stimulated
Time1* = initial temperature at arrival in blast chillers; Time2 = 6 h of chilling; Time3 = 12 h of chilling; Time4 = final hour of chilling
[a-d] Least squares means within a column with different letters are different ($P < 0.05$)
[x-z] Least squares means within a same row for the same variable and muscle with different letters are different ($P < 0.05$)

Table 29: Effects of carcass size, chilling rate, and electrical stimulation on tenderness attributes (WBSF, SSF) of the Longissimus lumborum (LL) plant A

	Heavy weight				Light weight			
	CC[1]		DC		CC		DC	
	NES[2]	ES	NES	ES	NES	ES	NES	ES
WBSF								
14 d	3.98cx	4.21bxyz	4.44bxyz	4.18cxy	4.27bxyz	4.05bxy	4.81bz	4.61byz
21 d	3.46bx	3.76bx	3.42ax	3.54bx	3.35ax	3.42ax	3.67ax	3.67ax
28 d	2.97axy	2.95axy	3.04axyz	2.91ax	3.32azyz	3.2azyz	3.55ayz	3.56az
35 d	3.2abx	2.93ax	3.3ax	2.94ax	3.28ax	3.3ax	3.39ax	3.23ax
n	22	22	20	20	20	20	20	20
SEM	0.22							
P-value	0.35							
SSF								
14 d	15.09cx	17.35cx	16.68cx	16.05bx	16.36cx	15.38cx	16.51bx	16.5bx
21 d	13.95bx	14.83cxy	15.15bcy	15.17by	13.48bx	14.61cxy	15.67by	14.25abxy
28 d	12.16abx	12.46ax	12.93abx	12.36ax	12.5bx	12.62bx	14.26abx	14.3abx
35 d	10.65axyz	11.08ayz	11.05ayz	12.49az	8.3ax	8.75azy	12.66az	12.37az
n	22	22	20	20	20	20	20	20
SEM	1.3							
P-value	0.44							

[1] CC = conventional spray chilling, DC = delay spray chilling

[2]ES = electrically stimulated; NES = non-stimulated

[a-d] Least squares means within a column with different letters are different (*P* < 0.05)

[x-z] Least squares means within a same row for the same variable and muscle with different letters are different (*P* < 0.05)