

POSTMORTEM INTERACTIONS OF CHILL TIME
AND ELECTRICAL STIMULATION ON
MUSCLE TEMPERATURE, pH, AND
OTHER BEEF QUALITY FACTORS

By

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CHAPTER I

INTRODUCTION

Producers, packers, retailers, and restaurateurs have struggled with inconsistency in beef palatability, specifically tenderness, for much of the 20th century and into the new millennium. Beef has the ability to offer palatability attributes not attainable by other protein sources. It has been shown that consumers recognize differences in tenderness and are willing to pay more for tender product (Boleman et al, 1997). The most recent National Beef Quality Audit (Smith et. al., 2005) identified lack of uniformity and consistency as the number one quality defect in the U.S. beef industry as perceived by end users. Length of time on a high-concentrate diet and other factors, such as wet or dry aging, has been shown to improve tenderness, and may lead to a more expensive product. The pork and poultry industries have a distinct advantage in that broiler and hogs are typically harvested at a young age in relation to beef, so maturity-related toughening is not a quality issue in these industries. The Beef Customer Satisfaction Study (Neely et al., 1998, 1999; Lorenzen et al., 1999; Savell et al., 1999) showed positive effects of tenderness on consumers' evaluation of taste. Therefore, the beef industry must continue to identify ways to improve tenderness and consistency of beef without increasing cost associated with processing.

First patented as a means of improving tenderness in beef carcasses by Harsham and Deatherage in 1951 (US Patent 5266481), electrical stimulation (ES) has been shown to have positive effects on tenderness, color, and quality. Prevention of cold shortening, physical disruption of muscle fibers, as well as increased proteolytic activity have all

been attributed to electrical stimulation's ability to increase tenderness. The condition in regard to pH and temperature, in which a carcass enters rigor mortis, can greatly affect lean color and quality. Electrical stimulation increases the rate of postmortem glycolysis, which causes rapid decline in pH. The resulting higher concentration of hydrogen ions likely affects the spatial arrangement of myoglobin, improving lean color.

The effect of cooling rate has also been shown to have an effect on tenderness. Hannula and Puolanne (2004) found a slow or low cooling rate, combined with pH, at the onset of rigor has a significant effect on tenderness. However, slow cooling rate decreases the efficiency of the harvest process and ultimately narrows the margin for economic gain sought by the beef packer. Koohmaraie et al. (1996) determined that meat does not toughen when sarcomere shortening is prevented. Therefore, it is the object of this experiment to determine the most effective combination of chilling rate and electrical stimulation that will allow packers to efficiently produce tender beef.

CHAPTER II

REVIEW OF LITERATURE

Carcass Chilling

The chilling of meat has been utilized since ancient times as a means of preservation. Prehistoric man discovered meat lasted longer when stored in a cool place or packed with snow and ice. As a result, animals would be killed and perishable items made during periods of cooler weather to prevent spoilage, sometimes packed with snow, ice, or salt (Aberle et al. 2001). Throughout time, ice was eventually harvested and stored. With much advancement in technology came the advent of refrigeration. Considered the father of refrigeration, John Gorrie obtained the first U.S. patent for refrigeration in 1851 (Gorrie, 1851). Mechanical refrigeration is accomplished through the fact that energy is gained and lost through the change of state of a gas. Gas is pressurized via mechanical compression until it turns to a liquid, which then passes through a condenser, removing heat generated during the change of state (Romans et al., 2001). Although not widely accepted by industry until the late 1800's, mechanical refrigeration is considered one of the most important advancements in technology for the meat packing industry. However, it was not until the 1930's when ice was finally phased out as the primary source of refrigeration.

Internal temperatures of cattle coming off the harvest floor are typically around 39° C (Aberle et. al., 2001). Conventional chill coolers, such as the one described above, can take 48 h or more to go from initial temperatures of around 39° C to a recommended temperature of 5 °C or less without affecting carcass quality (Aberle et al., 2001). With

the exception of poultry, the U.S. does not have any regulatory requirements for the endpoint chilling temperatures of livestock carcasses before processing. The meat industry operates on a low margin, high volume based system; as a result, the need for more rapid carcass cooling systems is needed to potentially increase profit. Blast chilling is one form of more rapid cooling where high-velocity air is used to aid in the dissipation of heat. However, due to the high amount of air circulation and low relative humidity present in conventional coolers, carcass shrink loss has been, and remains, a major economic factor in today's beef packing industry (Hamby et al., 1987). More commonly used today as a means of cooling and as an aid in controlling cooler shrink loss is the concept of spray chilling. Hippe et al. (1991), described spray chilling as the intermittent spraying of cold water on beef carcasses during the first 3-8 h postmortem. Hippe et al. (1991), as well as Allen et al. (1987) and Jones and Robertson (1988), all found a decrease in carcass shrink loss between 0.5 and 1.5 percent during the initial 24 h of postmortem chilling. Hippe et al. (1991) also found that leaner carcasses chilled faster but had higher shrink loss than fatter carcasses. However, even though some benefits have been shown by rapid cooling, the dissipation of heat can be achieved so efficiently it can have varying effects on quality.

Postmortem Biochemical Processes

The biochemical and structural changes that take place in the conversion of muscle to meat have a profound affect on meat quality. The removal of blood from the carcass, or exsanguination, marks the beginning of the conversion of muscle to meat, which occurs within the first 24 h postmortem. The circulatory system is responsible for the transport of nutrients and removal of waste products in the body. Exsanguination

prevents the circulatory system from functioning, which prevents any new energy in the form of glucose from being carried to the cell. In turn, anaerobic glycolysis, which is described by Berg et al. (2002) as the sequence of reactions that metabolizes glucose and pyruvate to ATP, takes over as the primary source of energy to cells. Bodwell et al. (1965) showed that at 0, 6, 12, 24, and 48 h postmortem glycogen content of the beef *longissimus dorsi* muscle was 56.7, 41.6, 30.4, 10.1, and 10.0 mmole glucose equivalents per gram. According to Berg et al. (2002) anaerobic glycolysis will yield 3 moles of ATP from one molecule glucose. Creatine phosphate reserves are the first energy source used to convert ADP back to ATP, however, creatine phosphate is rapidly depleted and ATP concentration declines (Romans et al., 2001). As a result of anaerobic glycolysis' utilization of glucose, lactic acid is built up in the muscle. Consequently, without a circulatory system to remove it, this build up of lactic acid decreases the pH of the muscle. The resulting lactic acid will continue to decrease the pH until glycogen is almost fully depleted or contractile proteins stop functioning as a result of low pH. The normal pH of muscle drops from nearly 7.0 at the time of harvest to the range of 5.5-5.7 within 24 h postmortem (Bechtel, 1986). The depletion of energy within the muscle allows myosin and actin to form tight bonds and prevents them from moving independently of each other causing the I-band to essentially disappear. These permanent cross-bridges are known as actomyosin (Savell et al., 2005). This process results in the conversion of muscle to meat as well as a condition known as *rigor mortis*.

Rigor Mortis (rigor) is described by Honikel et al. (1983) as the irreversible loss of extensibility of a whole bundle of muscle cells. Rigor forms a stiff and inextensible state that is easily differentiated from the soft and workable state that appears pre-rigor.

According to Hannula and Puolanne (2004) the onset of rigor in normal meat takes place around pH 5.7-5.8. Rigor occurs in three phases that ultimately are dependant on temperature and the rate at which anaerobic glycolysis proceeds. The faster a carcass is cooled, the slower glycolysis and the normal pH decline take place. Eventually, due to a lack of energy in the system, actomyosin cross-bridges are unable to be broken and stiffening of the muscle occurs. Delay, the first phase of rigor, is characterized by an extensible state of the muscle in which there is still sufficient amounts of ATP available to allow for relaxation of the muscle. During this phase, energy stores in the form of creatine phosphate, which allow for the phosphorylation of ADP into ATP, are still sufficient to allow relaxation of the muscle. However, creatine phosphate is depleted during this stage which causes a sharp decrease in ATP production. This loss of extensibility and significant depletion of stored energy marks the beginning of the onset phase. During the onset phase, muscles continue to increase in stiffness as more energy is depleted at the cellular level. This increase in stiffness continues until the completion phase. The completion phase of rigor mortis is characterized by the almost complete loss of extensibility. Creatine phosphate is depleted and no ATP can be formed for relaxation, which results in no extensibility and full rigor mortis (Aberle et al., 2001). Both rigor mortis and the postmortem pH decline are dependant on energy utilization. In addition, the rate at which a carcass is chilled has been shown to have a profound effect on energy utilization and ultimately tenderness (Hannula and Poulane, 2004; Honikel et al., 1983; Koohmaraie, 1996; Savell et al., 2005).

Tenderness

Tenderness, as defined by Davey (1983), is the amount of force required to slice a cross-sectional area of cooked meat across the muscle cells or fibers. Tenderness of meat, especially beef, has been studied perhaps more than any other attribute. Tenderness is determined by many factors including genetic makeup, physiological age, diet, growth rate, connective tissue, muscle shortening, as well as other antemortem and postmortem treatments. Genetic differences account for much of the variation among breed types of cattle; more specifically between *Bos indicus* and *Bos taurus* breeds of cattle. McKieth et al. (1985) and Crouse et al. (1987) both showed that *Bos indicus* cattle produce meat that is tougher than meat from *Bos taurus* cattle. This variation in tenderness has primarily been attributed to increased amounts of calpastatin within the *Bos indicus* breed (Koohmaraie et al., 1995b). Calpastatin is the endogenous inhibitor of the calpain system, which has been thought to be responsible for most if not all postmortem tenderization via degradation of key myofibrillar proteins (Koohmaraie, 1995b). Physiological age, as described by Aberle et al., (2001), is an expression of the degree of aging visible in the animal tissues. As an animal matures the amount of connective tissue (collagen) increases. Increased amounts of collagen and collagen cross-linking, result in decreased tenderness. This due to more heat-stabile web-like structures that retain higher residual strength after cooking (Aberle et al., 2001). Tenderness, juiciness, and flavor are three factors used to determine overall palatability. However, tenderness has been shown to be the most critical factor contributing to a pleasant eating experience. Boleman et al. (1997) showed that consumers recognize differences in tenderness and are willing to pay more for tender meat. Morgan et al. (1991) revealed

information in the National Beef Tenderness Study that confirmed the inconsistent tenderness of beef. The inconsistencies in beef, desire of consumers to eat healthier, as well as other more convenient protein sources have all led to a decrease in beef consumption. Lack of overall uniformity, consistency and tenderness of beef have been identified in the top ten quality defects in the US beef industry for the last 15 years (Smith et al., 2006). Koohmaraie (1995a) concluded inadequate or inconsistent beef tenderness is most likely the cause of consumer dissatisfaction. The USDA developed quality grades based on the idea that the increased presence of fat within the muscle, or marbling, is directly related to an increase in palatability. Marbling has been shown to have a positive effect on overall palatability. McBee and Wiles (1967) and Dolezal et. al. (1982) found that sensory acceptance increases with increased marbling. Likewise, Jones and Tatum (1994) reported differences in tenderness among quality grades. However, according to Wheeler et al. (1994) USDA quality grades only account for about 5% of the variation in beef tenderness. In addition, results from the 2005 National Beef Quality Audit revealed that just over 70% of carcasses graded fell into the lower one-third U.S. choice and U.S. select quality grades (Smith et al., 2006). Savell et al. (1987) concluded that no tenderness differences existed between “small” and “slight” marbling scores, but indicated shear force values were different when comparing a broader range of marbling scores. The desire of consumers to eat healthier poses a potential problem for the beef industry in that increased intramuscular fat has been shown to offer a more pleasurable eating experience (Smith et al., 2006). Several explanations related to the toughness of beef have been explored. Ease of fragmentation is defined by Aberle et al. (2001) as an expression of the ability of the teeth to cut across meat fibers. Muscles are held together

by a web-like structure surrounding the muscle fibers (endomysium), muscle fiber bundles (perimysium), and whole muscles (epimysium). These structures are made up primarily of collagen which results in “background toughness” associated with a particular muscle. Collagen is the most abundant protein in the animal and is directly related to muscle tenderness. The amount of collagen within a muscle depends on muscle activity. Muscles that undergo higher amounts of locomotion have greater amounts of collagen. In addition, Quali (1990) found greater degradation of myofibrillar structures in muscles with increased white muscle fibers as opposed to muscles with increased red fibers. Tenderness has also been shown to be affected by the degree of contraction caused by the chilling process (Savell et al., 2004).

Muscle Shortening

The chilling of carcasses within the first 24 h postmortem, when muscle is converted to meat, has been shown to be very critical with regards to beef tenderness (Honikel et al., 1983; Lochner et al., 1980; King et al., 2003). One negative effect associated with rapid chilling of beef is known as cold shortening. Cold shortening occurs when muscle with adequate concentrations of ATP contracts under cold conditions (Honikel et al., 1983). Locker and Haygard (1963) described cold shortening as a rapid reduction in temperature less than 14-19 °C before the onset of rigor mortis. Similarly, Honikel et al. (1983) found that cold shortening occurs below 15 °C depending on pH and ATP concentration. Cold shortening is dependant on ATP concentration in that, with sufficient ATP concentration muscle will contract and to a greater extent than normal living muscle. pH is directly related to the amount of energy in muscle, which in turn relates to the ability of the muscle to relax. Under the conditions described above, the

calcium pump of the sarcoplasmic reticulum does not function properly and calcium is built up. At this point, with sufficient ATP levels the muscle is able to contract to a great extent causing the I-band to disappear completely in some cases (Aberle et al., 2001). Conditions other than those aforementioned can also lead to shortening of the sarcomere. Heat and thaw rigor have also been attributed to sarcomere shortening. Heat rigor occurs when muscle is held at relatively high temperatures (up to 50 °C) during the onset of rigor, resulting in severe shortening in the muscle due to a rapid depletion of ATP (Aberle et al., 2001). Likewise, thaw rigor also leads to shortened sarcomere length but is much more severe. Aberle et al. (2001) describes thaw rigor as a type of shortening that occurs when pre-rigor muscle is frozen and then thawed. Under these conditions, contraction is caused by a sudden release of calcium into the sarcoplasmic reticulum. Muscles can contract up to 80 percent of their original length under thaw rigor conditions (Aberle et al., 2001). Thus, the packing industry must identify ways to combat the negative effects of properly chilling carcasses.

Electrical Stimulation

Electrical stimulation (ES) has been utilized in the beef industry as a means to increase tenderness, quality, and color. First patented by Harsham and Deatherage in 1951, ES is still being used in most major packing facilities in the U.S. today. However, due to a lack of interest by processors, this technology was not well accepted until the 1970's. The ability of ES to increase meat tenderness was extensively studied and eventually accepted in the late 1970's (Cross et al., 1979; McKeith et al., 1980, 1981; Savell et al., 1977, 1978a,b 1979). In early 1978, the LeFiell Company developed the first commercial ES system (Romans et al., 2001). In addition to increased tenderness,

electrical stimulation has also been attributed to improved lean color (McKeith et al., 1980, 1981). These theories are widely attributed to three main factors including increased postmortem glycolysis, physical disruption of muscle fibers, and increased proteolytic degradation (Savell et al., 1979).

Effects of Electrical Stimulation on Tenderness

Over two decades had passed after the initial patents were granted for use of ES to improve tenderness in beef carcasses before researchers in New Zealand sought to improve tenderness of lamb carcasses through ES. The aforementioned effects, of what is now known as cold shortening, was the main focus of their research. Increased postmortem glycolysis, resulting from ES, was reported by Carse (1973) to effectively prevent cold shortening. As mentioned earlier, cold shortening takes place when pre-rigor muscle with adequate energy reserves is exposed to temperatures below 15°C. Electrical stimulation has been shown to increase postmortem glycolysis rate, thus decreasing ATP concentration causing muscle to lose the ability to contract when temperatures decrease under normal cooling conditions. When pre-rigor muscle is exposed to cold temperatures the sarcoplasmic reticulum and mitochondria are inhibited in their ability to bind calcium ions. This, in turn, leaves excess intercellular calcium, which, in the presence of ATP causes continual contraction of the muscle fibers. Electrically stimulated post-rigor muscle does not incur cold shortening due to a lack of ATP. This increase in postmortem glycolysis also causes an immediate drop in pH. Following this drop in pH there is temperature dependant acceleration of glycolysis which leads to early rigor mortis. Reaching rigor mortis early or before exposure to refrigeration temperatures will also prevent shortening of the sarcomeres. However,

there is evidence that the ability of ES to increase tenderness can not be attributed solely to increase in postmortem glycolysis. Devine et al. (2001) found no difference in initial shear force values, rate of change of shear force values, or final shear force values when comparing electrically stimulated to non-stimulated animals chilled at the same temperature. This conclusion lends evidence that suggests there are other means by which ES increases tenderness.

The ability of ES to increase tenderness via physical disruption of muscle fibers has also been attributed to ES. Dutson et al. (1977) first concluded ES of muscle resulted in changes in the ultrastructure of beef. Physical alteration (cold shortening) was found to have significant effects on tenderness even prior to industry-wide acceptance of ES (Marsh et al., 1974). Savell et al. (1978a) also theorized that ES causes massive contraction which could in turn disrupt muscle fibers. In addition, Takahashi et al. (1984, 1987) determined that 50-60 Hz with 500 V, 40 min postmortem resulted in physical alteration of the muscle structure as well as increased tenderness. In contrast, McKeith et al. (1980) found no physical disruption of muscle fibers directly related to supercontracture from ES. Similarly George et al. (1980) attributed the physical disruption to denaturation of sarcoplasmic proteins due to increased proteolytic activity.

Electrical stimulation's ability to increase tenderness, although not completely understood, has also been associated with its ability to increase the activity of proteolytic enzymes (Savell et al., 1978b, 1979; Dutson et al., 1980). Aging is described by Hwang et al. (2003) as the process of meat becoming more tender over time and involves specific degradation of muscle proteins. Ho et al. (1996) concluded ES accelerates the degradation of the cytoskeleton proteins titin, nebulin, desmin, and troponin-T in

postmortem muscle. In addition, wide I-band fractures appeared sooner postmortem in electrically stimulated muscle (Ho et al., 1996). Dransfield et al. (1992) as well as Uytterhaegen et al. (1992), both showed conclusions of stimulated activity of some proteolytic enzymes, including μ -calpain. Koohmaraire and Geesnick (2006) concluded that most, if not all, postmortem tenderization is due to the calpain system, specifically μ -calpain. As cellular membranes are disrupted, calcium is leached into intercellular spaces where the calcium induced calpain system can begin degradation of key myofibrillar proteins. The significant drop in pH, as well as the ability to physically disrupt cellular integrity, is the basis for electrical stimulation's ability to increase proteolytic activity. Watanabe and Devine (1996) presented evidence that titin and nebulin are degraded further as pH lowers. This study supports the ability of electrical stimulation to increase tenderness by decreasing the amount of time to achieve ultimate pH. This corresponds to earlier research conducted by Savell et al. (1978b) and Dutson et al. (1980) that suggests lysosomal membranes could be disrupted physically and/or chemically to increase proteolytic activity. Any one or combination of the aforementioned theories regarding ES could be responsible for increasing tenderness. In addition to its ability to increase tenderness ES has also been shown to have positive effects on other carcass characteristics.

Effects of Electrical Stimulation on Quality and Lean Color

Electrical stimulation has been collaboratively shown to improve both lean color and texture (Savell et al., 1978a,b, 1979; McKeith et al., 1981; Roeber et al., 2000). The improvements in color and texture have been associated with an increase in postmortem glycolysis due to increased muscle activity via ES. In an anaerobic environment lactic

acid is built up as an end product of glycolysis as opposed to pyruvate in an aerobic environment. This increase in glycolytic activity causes an increase in lactic acid accumulation in muscle, ultimately lowering the pH more rapidly. It was reported by Munns and Burrell (1965) that an ultimate pH of 6.0 or higher included 90% of dark lean color. Ashmore et al. (1973) concluded mitochondrial respiration at higher pH values remains high. Under these conditions myoglobin, the protein responsible for meat color, is deoxygenated or only partially oxygenated due to higher oxygen utilization by surviving enzyme systems. As pH of lean tissue is decreased permeability to oxygen is increased resulting in a brighter more cherry red color. This bright cherry red color is related to the state of oxygenation of myoglobin; as myoglobin nears its isoelectric point (5.5), where it would contain no net charge, its binding ability decreases. Therefore, at higher pH levels, myoglobin will bind more water causing muscle fibers to expand leaving less space between them for free water to reflect light resulting in a darker appearance (Lawrie, 1974). The relationship of ultimate pH and muscle color has been well documented (Watanabe et al., 1995; Jeremiah et al., 1991). The spatial arrangement of myoglobin, in either ferrous (Fe^{++}) or ferric (Fe^{+++}), determines the color it will appear. In the less reduced ferrous state, the internally bound iron molecule can transition from the oxygenated to the deoxygenated state. In the presence of oxygen myoglobin will assume the oxygenated state and appear bright cherry red, whereas in the deoxygenated state myoglobin will appear purple in color. In contrast, the ferric state of myoglobin, being more oxidized, will appear brown and is known as metmyoglobin. In addition, Wulf et al. (1997) reported muscle pH is highly correlated with CIE L^* ($L^*=0$ yields dark and $L^*=100$ indicates light), a^* (negative values indicate green while positive values

indicate red), b^* (negative values indicate blue and positive values indicate yellow). This study showed that following low voltage ES lower pH values resulted in muscle that was more light than dark (higher L^*), more red than green (higher a^*), and more yellow than blue (higher b^*), and also had higher taste panel tenderness ratings. Roeber et al., (2000) also reported improvements in L^* , a^* , and b^* values associated with ES. In addition to these positive effects, ES has also been shown to decrease incidence of heat ring.

Heat ring is a color defect associated with graded levels of pH across muscle areas from the surface of the carcass inward particularly in carcasses with a relatively thin layer of fat (Romans et al., 2001). Glycolysis is slowed in the faster chilling external portions of the carcass this results in slower pH decline and longer delay until onset of rigor mortis. In contrast, the inner portion of the muscle, being better insulated allows for a slower, more thorough drop in pH resulting in lighter color. Electrical stimulation has been shown to decrease incidence of heat ring through acceleration of pH decline throughout the muscle.

Conclusion

Sufficient evidence has been compiled to conclude ES, in combination with accelerated chilling, can efficiently and effectively increase production and decrease the need for larger facilities while having no detrimental effect on end product quality or palatability. The beef industry must continue to improve efficiency and effectiveness of harvest facilities, thus, the subsequent research was conducted to determine what combination of ES and accelerated chilling could accomplish this goal.

CHAPTER III

POSTMORTEM INTERACTIONS OF CHILL TIME AND ELECTRICAL STIMULATION ON MUSCLE TEMPERATURE, pH, AND OTHER BEEF QUALITY FACTORS

ABSTRACT

The objective of this study was to determine postmortem effects of muscle temperature, electrical stimulation, and muscle pH on beef quality. Two-hundred finished cattle were selected based on common breed type, weight, and estimated fat thickness. Immediately following harvest, carcasses were randomly selected to be assigned to one of eight electrical stimulation (ES) x postmortem chilling time (23 h or 30 h) combinations: 1) Control: no ES/23 h; 2) no ES/30 h; 3) 100 V ES/23 h; 4) 100 V ES/30 h; 5) 250 V ES/23 h; 6) 250 V ES/30 h; 7) 400 V ES/23 h; 8) 400 V ES/30 h. Electrical stimulation was applied through brisket probes coming in contact as carcasses passed. The appropriate voltage was applied at 1.67 amps for 63 s in 1 s intervals with 1 s in between ES pulses. Temperature and pH measurements were taken prior to ES and at 3, 6, 12, and 23 h postmortem. CIE L*, a*, and b* measurements, as well as ribeye area and preliminary yield grade, were collected using the vision grading system used by National Beef Packing. Mean marbling score did not differ ($P > 0.05$) between chilling times or electrical stimulation levels from non-stimulated controls. Mean values for CIE L*, and a* of lean color were more light and more red ($P > 0.05$) for the 30 h chilled carcasses as compared to carcasses chilled 23 h in the non-stimulated group. No significant color differences ($P > 0.05$) were found between ES levels. Slice shear force

values were higher for non-stimulated controls at 7 and 14d than for ES sides. Longissimus muscle pH was generally higher ($P < 0.05$) for non-stimulated controls as compared to ES treatments. Differences in temperature were not different between 23 and 30 h chilled carcasses. Chilling of carcasses for 23 h versus 30 h showed no detrimental effects on quality. In addition, electrical stimulation increased tenderness and improved lean color when compared to non-stimulated control sides. The current study revealed evidence that could aid the beef industry in increasing throughput and possibly increasing profit.

INTRODUCTION

The packing industry operates on a low margin, high volume basis and relies on the ability to harvest, refrigerate, and process large numbers of cattle to remain profitable. Any increase in efficiency, or decrease in processing time, within the confines of the harvest facility can ultimately increase profits and decrease the need to build larger facilities. Chilling is one of the most costly processes incurred by the packing industry. Accelerated chilling has been shown to reduce cooler shrink, increase perception of marbling, and reduce the time of aging to achieve an acceptable product (Aalhus et al., 2001). However, advanced chilling systems have also been shown to cause cold shortening, produce darker lean color, and increase incidence of heat ring formation. Fortunately, these negative effects can be prevented or eliminated through the use of ES. Electrical stimulation has been shown to prevent cold shortening, produce improved lean color, prevent formation of heat ring, and shorten the aging period required to produce a quality product (Aberle et al., 2001). Savell et al. (1978b) reported that electrical stimulation has the ability to accelerate post-mortem aging which would thereby decrease

the aging time required to obtain a more tender product. Boleman et al. (1997) showed that consumers recognize differences in tenderness and are willing to pay more for tender meat. Thus, this experiment was conducted to evaluate the most effective use of ES in combination with chilling rate to efficiently and effectively produce quality beef.

MATERIALS AND METHODS

Sample Collection

Finished cattle (n = 200) were selected based on common breed type, weight, and estimated fat thickness. Cattle were harvested using humane methods at National Beef Packing in Dodge City, KS. Immediately following harvest, paired carcass sides (n = 400) were randomly selected to be assigned to one of eight electrical stimulation (ES) postmortem chilling time (23 h or 30 h) combinations (Table 1): 1) 0 V ES/23 h; 2) no ES/30 h; 3) 100 V ES/23 h; 4) 100 V ES/30 h; 5) 250 V ES/23 h; 6) 250 V ES/30 h; 7) 400 V ES/23 h; 8) 400 V ES/30 h. Table 1 represents the number of carcasses in each treatment. Temperature and pH measurements were taken prior to ES. Electrical stimulation was applied at each voltage level through spring loaded probes that came in contact as the carcass side passed through a stimulation chamber designed and made by National Beef Packing Inc. Each probe delivered the appropriate voltage at 1.67 amps for 63 s in 1 s intervals with 1 s between ES pulses. Immediately following each ES application, each carcass side passed through a steam pasteurization cabinet. One side from each carcass was put into either a 23 h or 30 h cooler where additional measurements were obtained. Upon removal of the carcass sides from the cooler at their designated chill time, each side was weighed, ribbed and given sufficient time (20 m) to bloom before USDA quality grade factors were collected by two official USDA graders.

Additional data were collected using the vision grading system camera (RMS) operated by a National Beef Packing Co. employee. The RMS camera collected preliminary yield grade, L^* , a^* , b^* , and ribeye area. Graded weight was also collected for determination of shrink loss. Lean color was evaluated through the camera in CIE L^* ($L^*=0$ yields black and $L^*=100$ indicates white), a^* (negative values indicate green while positive values indicate red), and b^* (negative values indicate blue and positive values indicate yellow). Immediately following carcass data collection, ribeye samples (IMPS # 112) approximately 10 cm long were excised from the 12th/13th rib interface of right and left sides. The samples were removed using a 6 in boning knife by holding the knife in a vertical position, following the curvature of the 10th, 11th, and 12th rib. An incision was then made 0.5 in lateral of the longissimus costarum vertically down the 10th, 11th, and 12th rib. The final incision was made by cutting vertically along the dorsal processes of the thoracic vertebrae of the 10th, 11th, and 12th ribs. The samples were labeled, packaged and transported to Oklahoma State University for further analysis.

Temperature and pH Determination

Temperature measurements were obtained using a Versa Tuff 386 type T thermocouple (Model 38653-T, Atkins Technical INC, Gainesville, FL), at 0, 3, 6, 12, and 23 h postmortem. pH measurements were taken using a Model IQ 140 pH meter (and taken at the same time as temperature measurements. All measurements were taken by making an incision through the subcutaneous fat and exterior layer of connective tissue followed by penetration of each probe into the *longissimus dorsi muscle* approximately 7 cm posterior to the 13th rib. Initial (0 h) measurements were taken immediately following harvest, prior to ES.

Postmortem Aging of Samples

Upon arrival at the Oklahoma Food and Agriculture Products Center located on the Oklahoma State University campus, longissimus samples were fabricated on a saw into steaks (2.54 cm). Samples were assigned to one of two aging periods, 7 or 14 d. Samples were then vacuum packaged in 8 x 10 vacuum pouches (Prime Source, 3 Mil. high barrier) and stored in the dark at refrigeration temperatures for the specified aging period. Following aging, samples were blast frozen until slice shear force analysis was conducted.

Shear Force Determination

Tenderness was assessed using the slice shear force method (Shackelford et. al., 1999). Steaks were tempered for 24 h at 4°C prior to cooking. Steaks from each ES level and chill period were randomized, to avoid variations in cooking. Steaks were broiled in an impingement oven (Lincoln Impenger, Model 1132-00-A, Fort Wayne, IN) at 180°C to a medium degree of doneness or a final temperature of 70°C. Final temperatures were monitored using a Versa Tuff 386 type T thermocouple (Model 38653-T, Atkins Technical INC, Gainesville, FL). Following cooking, steaks were allowed to cool for 24 h at 4° C on plastic trays, approximately 7.6 cm apart on an upright tray rack, to allow for proper dissipation of heat. Slice shear force measurements were determined by shearing a slice 1 cm thick, 5 cm long parallel to muscle fiber orientation, on a Universal Instron Testing Machine (Model 4502, Instron, Canton, MS) at a cross head speed of 500 mm/min. Each slice was taken approximately 6-7 cm from the lateral end of the steak by squaring 1-2 cm off the lateral end, then placing the remaining steak portion in a pre-

measured sizing box to obtain a 5 cm portion. The 5 cm portion was then moved to a 45° slice box where a double bladed knife was used to excise a slice, parallel to muscle fiber orientation, 1 cm long and 5 cm thick. The slice was then sheared perpendicular to the muscle fibers to determine kg of force required to shear through the sample.

Statistical Analysis

Treatments (electrical stimulation, chilling time) were arranged in a 2 x 4 factorial, completely randomized design. The model included electrical stimulation, chilling time and the interaction term as the main unit factors. Data were analyzed using the GLM procedures of SAS (SAS Inst., Inc., Cary, NC). Mean differences for least squares means were determined using Tukey's procedure ($\alpha = 0.05$) when the model demonstrated a treatment effect ($P < 0.05$). The frequency tough vs. tender steaks, was analyzed using the Frequency Procedures of SAS (SAS Inst. Inc., Cary, NC). Differences between percentages for aging periods (7 d, 14 d) and chilling times (23 h, 30 h) were determined by calculating the chi-squared statistic.

Results and Discussion

Marbling

Effects of chilling time and ES on marbling score of paired beef sides are presented in Figure 1. Although marbling score for the 23 h chill treatment had the highest numerical LS mean, no significant marbling differences existed between chilling times. Furthermore, marbling score for carcasses chilled for 23 h revealed no significant differences ($P > 0.05$) existed between ES levels. In addition, other studies have also shown ES had no significant effect with regard to increasing marbling score (Savell et al., 1978b; Davis et al., 1981; Crouse et al., 1983).

Longissimus Muscle Color

Mean CIE L* values for chilling time and ES level are presented in Figure 2. Within the ES control group (0 V), L* values were significantly higher ($P < 0.05$) for longissimus samples chilled for 30 h compared to 23 h counterparts. Although no significant difference ($P > 0.05$) was detected, the longissimus muscles for ES sides displayed higher L* values than those from non-ES controls within the same chilling time. The influence of ES and chilling time on mean CIE a* values are presented in Figure 3. Similar to L* values, a* values were also significantly higher ($P < 0.05$) for the 30 h chill than for 23 h chill within in the ES control group (0 V). Although not significantly different ($P > 0.05$), a* values were higher for the 400 V ES/30 h chill group than any other treatment. These results are similar to findings by Roeber et al. (2000) that concluded a* values are higher, or redder, for ES beef muscles as opposed to non-stimulated control muscles. Mean CIE b* values for ES levels and chilling times are presented in Figure 4. No significant differences ($P > 0.05$) existed between ES levels or chilling times. Although not significantly different, mean b* values for 400 V/23 h chill sides were numerically lower, suggesting a more blue color. These results are not concurrent with previous research that concluded ES significantly increased b* values over non-stimulated muscles (Roeber, et al., 2000; Savell et al. 1978a,b). Furthermore, Wulf et al. (1999) found that a* and b* values continued to increase, or become redder and less blue, for 75 min after ribbing. This could explain why no significant differences were detected, as bloom time in the present study was 15-20 min. In addition, Clydesdale and Francis (1971) proposed that lower b* values are positively associated with increased

deoxymyoglobin resulting in darker colored lean. As previously mentioned, ES has shown the ability to increase b^* values resulting in lighter colored lean. Higher b^* values, indicating more yellow, have also been associated with increased tenderness (Wulf et al., 1997).

Shear Force

Effects of chilling time and aging period on slice shear force values of ES longissimus muscles are presented in Figure 5. No significant difference ($P > 0.05$) existed between chilling times within ES levels.

Effect of chilling time and ES level on slice shear force values of longissimus muscles aged for 7 d are presented in Figure 6. Slice shear force values were higher ($P < 0.05$) for non-stimulated control sides than for stimulated sides irrelevant of chilling time, with the exception of the 400 V/30 h treatment, which was not different ($P > 0.05$) from the control (0 V) 30 h chill. Steaks in the 100 V/23 h chill treatment had the lowest shear force values, however this treatment was only different ($P < 0.05$) from the 400 V/30 h treatment between ES levels. No other differences ($P > 0.05$) existed between ES levels.

Figure 7 presents the effects of chilling time and ES level on slice shear force values of longissimus muscles aged for 14 d. No differences ($P > 0.05$) in slice shear force values existed between chilling times or ES levels. Slice shear force values from the 100 V/23 h, 100 V/30 h, 250 V/23 h, and 400 V/30 h chill treatment were lower ($P < 0.05$) than non-stimulated controls. These results are similar to most previous studies on ES (Cross et al., 1979; McKeith et al., 1980, 1981; Savell et al., 1977, 1978a,b 1979). No differences ($P > 0.05$) existed between the 400 V/23 h, 250 V/30 h, and the 30 h non-stimulated control (0 V) sides.

The comparison of tough vs. tender steaks from different chill times and aging periods of non-electrically stimulated (0 V) beef carcasses is presented in Figure 8. Percentages of tender steaks were not different ($P > 0.05$) between chilling times when steaks were aged for either 7 or 14 d. However, the percentage of tender steaks in the 30 h/14 d treatment was 36% higher than the percentage of tender steaks from the 23 h/7 d treatment. This is in agreement with previous research which suggests proteolysis of myofibrillar proteins during aging contributes the tenderization of meat during refrigerated storage (Davey, 1983; 1988, 1994, 1995a; Taylor et al., 1995). The comparison of percentages of tough vs. tender steaks from different chill times and aging period of carcasses stimulated with 100 V is presented in Figure 9. The percentage of tender vs. tough steaks was not different ($P > 0.05$) between chilling times when steaks were aged for either 7 or 14 d. In addition, the percentage of tender steaks from the 23 h/14 d treatment was higher ($P < 0.05$) than that of the 30 h/ 7 d treatment. Figure 10 presents the percentage of tender vs. tough steaks of carcasses stimulated with 250 V from different chilling times. No significant differences ($P > 0.05$) existed between chilling times from steaks aged for 7 or 14 d. Although not significantly different, the percentage of tender steaks from the 14 d aging periods was numerically higher than the percentage of tender steaks aged for 7 d . This is also in agreement with the previous studies that concluded increased tenderness due to proteolysis of key myofibrillar proteins during aging (Davey, 1983; 1988, 1994, 1995a; Taylor et al., 1995). Comparison of tender vs. tough steaks of carcasses stimulated with 400 V from carcasses chilled for either 23 or 30 h and aged for either 7 or 14 days is presented in Figure 11. The percentage of tender steaks for carcasses in the 30 h/14 d treatment was significantly

higher ($P < 0.05$) than steaks from the 30 h/7 d. The percentage of tender steaks from the 30 h/14 d treatment was the highest, however, no significant differences were found.

Shrink Loss

The influence of chill time on shrink loss of electrically stimulated beef carcasses is represented in Figure 12. There were no significant differences ($P > 0.05$) in shrink loss between chilling times. Additionally, although shrink loss for the 250 V/ 23 h chill was the highest, there were no significant differences ($P > 0.05$) between ES levels.

pH and Temperature Measurements

Comparison of postmortem pH decline of ES longissimus muscle (LD) for carcasses chilled 23 h is presented in Table 2. Initial (0 h) pH measurements were taken before ES for all carcasses and averaged. Electrically stimulated carcasses had lower ($P < 0.05$) pH values than did non-stimulated controls. Additionally, pH values for carcasses in the 250 and 400 V treatments were lower ($P < 0.05$) than carcasses in the 100 V treatment. This is in agreement with research conducted by and George et al. (1980) that concluded ES causes a rapid decline in pH. Carcass pH values at 6 h were numerically higher than at 3 h for the 250 and 400 volt treatments. This could be due in part to decreasing temperatures, which has been concluded to slow postmortem biochemical processes (Hannula and Puolane 2004). Additionally, carcasses in the 250 V treatment had lower LD muscle pH values than did carcasses in the 100 V and non-stimulated treatments. Similar to 3 h pH values, 12 h pH values of ES sides were lower ($P < 0.05$) than non-stimulated control sides.

Comparison of postmortem pH decline of ES beef LD for carcasses chilled 30 h is presented in Table 3. At 3 h postmortem, ES beef carcasses had lower ($P < 0.05$) pH

values than non-stimulated controls. In addition, carcasses in the 250 V treatment had lower ($P < 0.05$) pH values than 100 V and 400 V treated carcasses. Comparison of pH at 6 h and 12 h, illustrated similar results to those at 3 h in that pH values for ES sides were lower ($P < 0.05$) than the non-stimulated control sides.

Comparisons of postmortem temperature decline of ES beef LD muscles for carcasses chilled 23 h are presented in Table 4. At 3 h postmortem temperature measurements for the 400 V treatment were higher ($P < 0.05$) than non-stimulated control sides. However, at 6 h and 12 h postmortem, non-stimulated carcasses displayed higher ($P < 0.05$) LD temperatures than carcasses in the 400 V group. Additionally, at 23 h, LD temperatures were not different for carcasses in the 400 V treatment when compared to non-stimulated control sides. Bendall (1973) and Davey and Gilbert (1974) both concluded reaching 10°C before 10 h, or before attaining a pH of 6.0, prevented cold shortening. In the current study, LD temperatures from carcasses in all ES treatments reached below 45° C in 12 h. This information, in addition to pH decline and shear force values, suggest chilling carcasses for 23 h has no negative effects when compared to chilling carcasses for 30 h.

Comparisons of postmortem temperature decline of ES beef LD muscles for carcasses chilled 30 h are presented in Table 5. Temperature measurements at 3 h for carcasses in the 400 volt treatment were higher ($P < 0.05$) than any other treatment. However, at 6 h postmortem, no differences ($P > 0.05$) were found between any treatment. Temperature measurements at 12 h postmortem were higher ($P < 0.05$) for 250 V and 400 V sides when compared to non-stimulated control sides. Conversely,

temperature measurements for 23 h revealed no differences ($P > 0.05$) between non-stimulated control sides and the 250 V and 400 V treatments.

Implications

Electrical stimulation in combination with 23 h chill showed no detrimental effects on quality when compared to ES carcasses chilled for 30 h. In addition, ES showed some ability to increase tenderness over non-stimulated control sides when aged for 7 or 14 d. As in previous studies, electrically stimulated carcasses, regardless of the voltage level applied, displayed lighter, more desirable lean color that was redder and more desirable than non-stimulated carcass sides. In all cases, although not significantly different ($P < 0.05$) the 23 h chilled carcass sides displayed slightly higher shrink losses than their 30 h chilled counterparts. The milder 30 h chilling procedure allows for less carcass shrink especially when 100 or 250 V electrical stimulation treatment is applied. The current study revealed evidence that under controlled conditions carcasses can be chilled for 23 h without negatively affecting quality, which could aid the beef industry in increasing throughput and possibly increasing profit.

Table 1. Carcass side selection matrix

Electrical Stimulation (V)	Chill Time, h	
	23	30
0	50	50
100	50	50
250	50	50
400	50	50
Total Sides	200	200

Table 2. Comparison of postmortem pH decline of electrically stimulated (ES) beef longissimus muscle for carcasses chilled 23 h

ES Level	h, postmortem			
	0 ¹	3 (n = 101)	6 (n = 97)	12 (n = 105)
0	7.3	6.11 ^a	5.83 ^a	5.75 ^a
100	7.3	6.31 ^b	5.79 ^a	5.64 ^b
250	7.3	5.64 ^c	5.69 ^b	5.61 ^b
400	7.3	5.74 ^c	5.78 ^{ab}	5.60 ^b
SEM		0.1605	0.1637	0.1574

^{a, b, c} Means, within columns, that do not share a common superscript are significantly different ($P < 0.05$)

¹0 h pH= average of all carcasses prior to ES

Table 3. Comparison of postmortem pH decline of electrically stimulated (ES) beef longissimus muscle for carcasses chilled 30 h

ES Level	h, postmortem			
	0 ¹	3 (n = 98)	6 (n = 104)	12 (n = 105)
0	7.3	6.01 ^a	6.07 ^a	5.70 ^a
100	7.3	5.89 ^b	5.92 ^b	5.68 ^b
250	7.3	5.71 ^c	5.82 ^c	5.62 ^b
400	7.3	5.83 ^b	5.81 ^c	5.63 ^b
SEM		0.0602	0.0613	0.0634

^{a, b, c} Means, within columns, that do not share a common superscript are significantly different ($P < 0.05$)

¹0 h pH= average of all carcasses prior to ES

Table 4. Comparison of postmortem temperature decline of electrically stimulated (ES) beef longissimus muscle for carcasses chilled 23 h

ES Level	h, postmortem				
	0 ¹	3 (n = 100)	6 (n = 97)	12 (n = 101)	23 (n = 102)
0	102.2	74.95 ^a	65.53 ^b	44.72 ^{ab}	32.20 ^b
100	102.2	75.32 ^{ab}	66.30 ^a	42.11 ^{bc}	31.05 ^c
250	102.2	77.08 ^{ab}	63.30 ^b	45.93 ^a	33.45 ^a
400	102.2	76.11 ^b	60.75 ^c	41.10 ^c	31.76 ^b
SEM		0.6156	1.0926	1.1238	0.4990

^{a, b, c} Means, within columns, that do not share a common superscript are significantly different (P < 0.05)

¹0 h temperature= average of all carcasses prior to ES

Table 5. Comparison of postmortem temperature decline of electrically stimulated (ES) beef longissimus muscle for carcasses chilled 30 h

ES Level	h, postmortem				
	0 ¹ (n = 186)	3 (n = 97)	6 (n = 105)	12 (n = 107)	23 (n = 102)
0	102.2	72.30 ^a	60.00 ^a	46.40 ^b	33.80 ^a
100	102.2	72.00 ^a	59.80 ^a	42.15 ^c	32.68 ^b
250	102.2	73.20 ^a	59.82 ^a	49.02 ^a	33.86 ^a
400	102.2	75.90 ^b	59.79 ^a	51.03 ^a	34.04 ^a
SEM		0.8536	0.0402	0.0398	0.0408

^{a, b, c} Means within, columns, that do not share a common superscript are significantly different ($P < 0.05$).

¹0 h temperature= average of all carcasses prior to ES

Figure 1. Effect of chilling time on marbling score of electrically stimulated paired beef carcasses.

^a Marbling Score (NBP Gold Standard): 400 = Small 00

No marbling differences ($P > 0.05$) existed between chilling times or electrical stimulation levels.

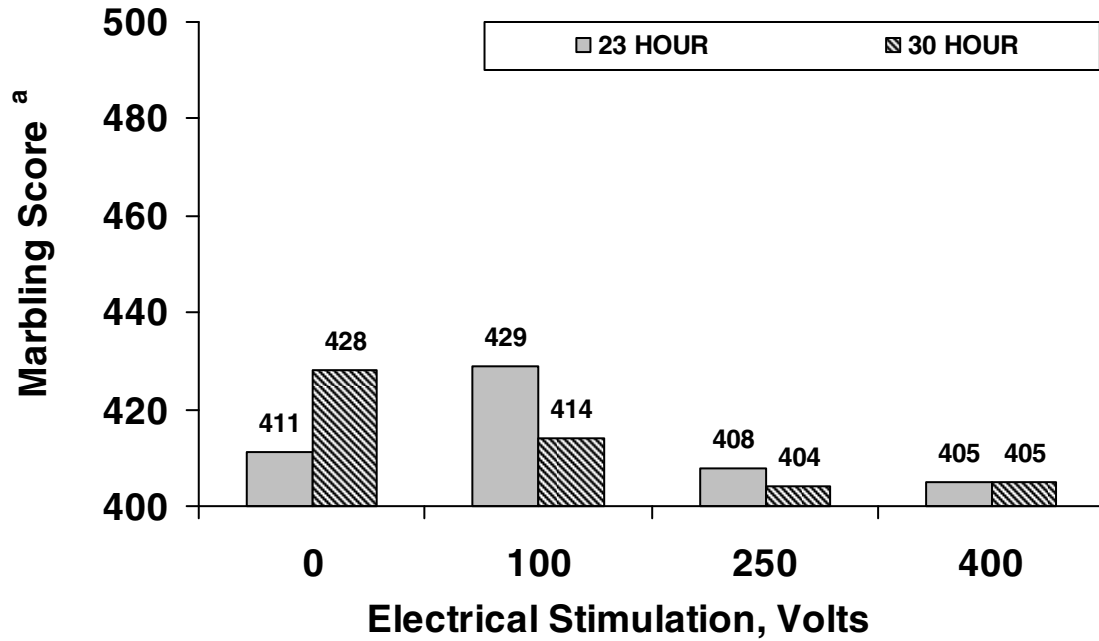


Figure 2. Influence of chilling time of ribeye lean color lightness (L*) of electrically stimulated beef carcasses.

^{a, b}Chill time means, within an electrical stimulation level, with different letters were statistically ($P < 0.05$) different.

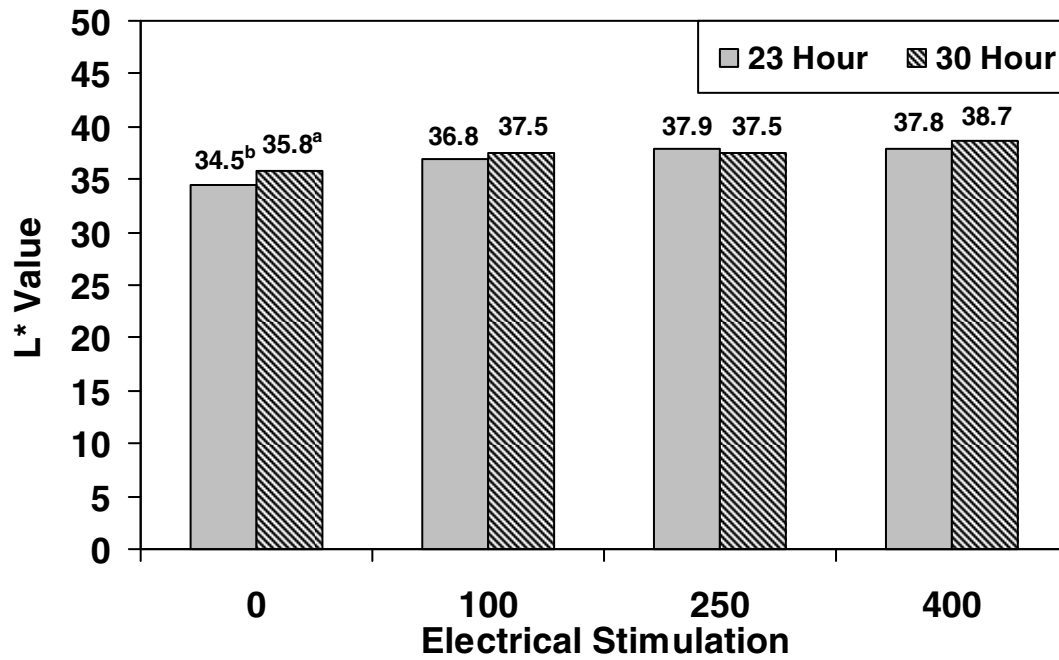


Figure 3. Influence of chill time on redness (a^*) of longissimus dorsi muscle of electrically stimulated beef carcasses.

^{a, b}Chill time means, within an electrical stimulation level, with different letters were statistically ($P < 0.05$) different.

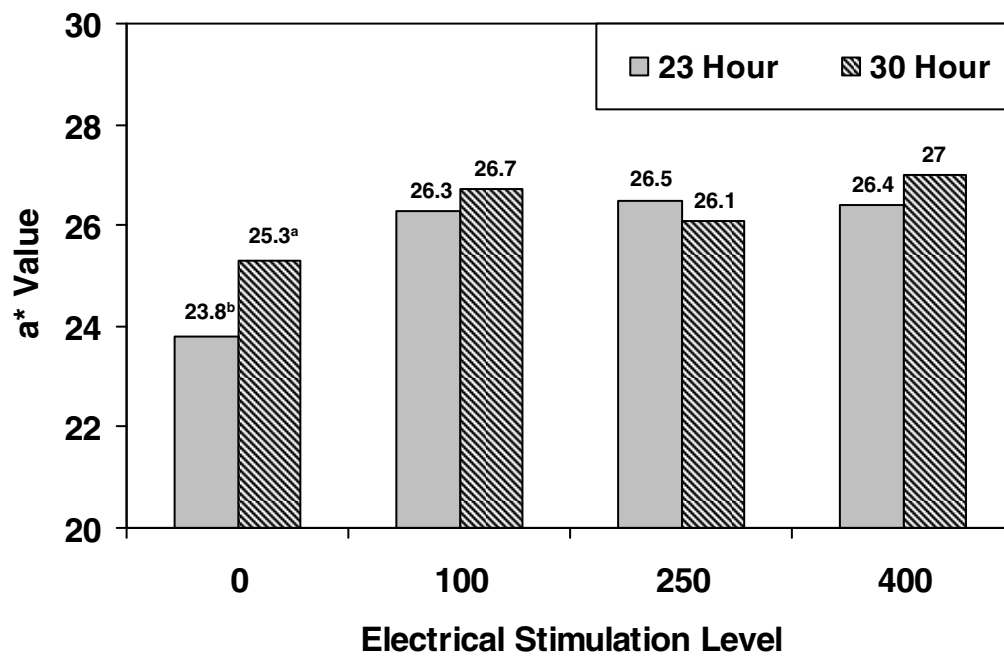


Figure 4. Influence of chill time on b* values of longissimus dorsi muscle of electrically stimulated beef carcasses
No b* value differences ($P > 0.05$) existed between chilling times within ES levels

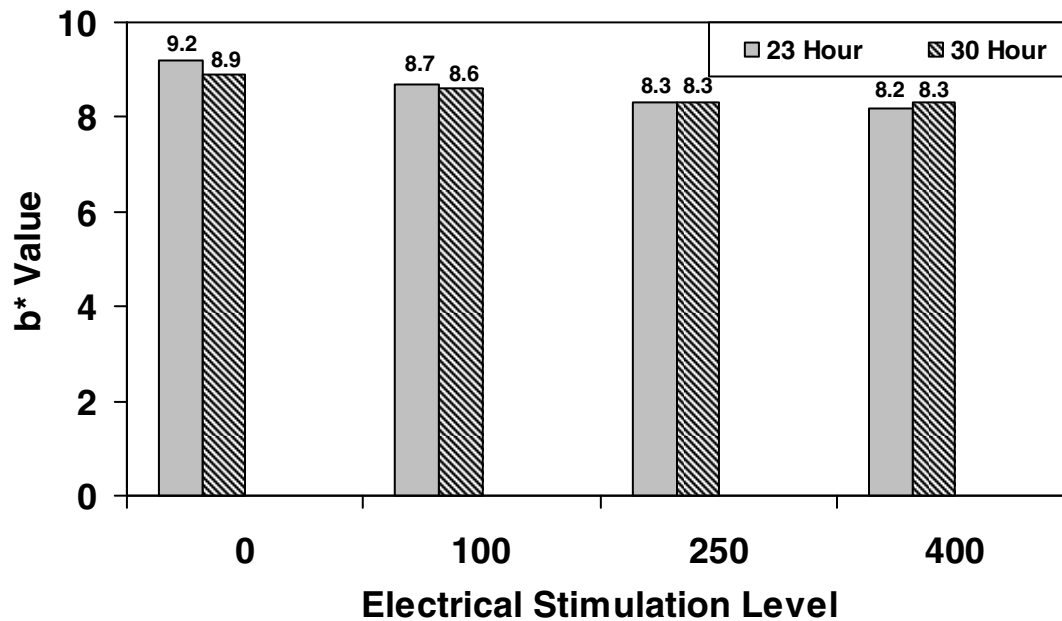


Figure 5. Effect of chilling and postmortem aging times on slice shear force values (kg) of electrically stimulated beef carcasses
 No marbling differences ($P > 0.05$) existed between chilling times within electrical stimulation levels.

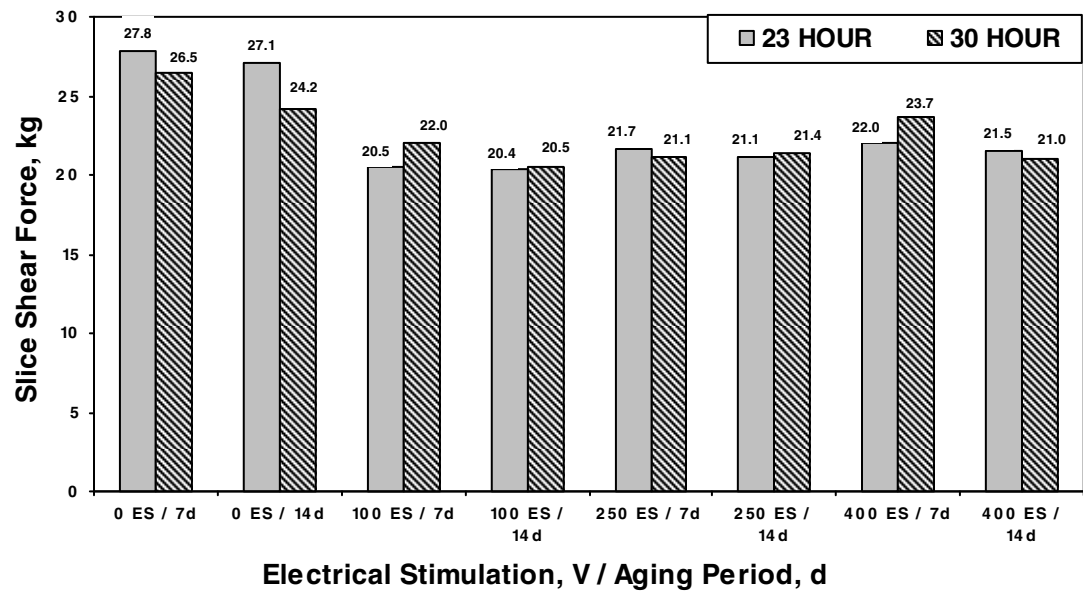


Figure 6. Effect of chilling and postmortem aging time (7 d) on slice shear force values (kg) of electrically stimulated beef carcasses
^{a,b,c,d} Means lacking common superscripts differ ($P < 0.05$)

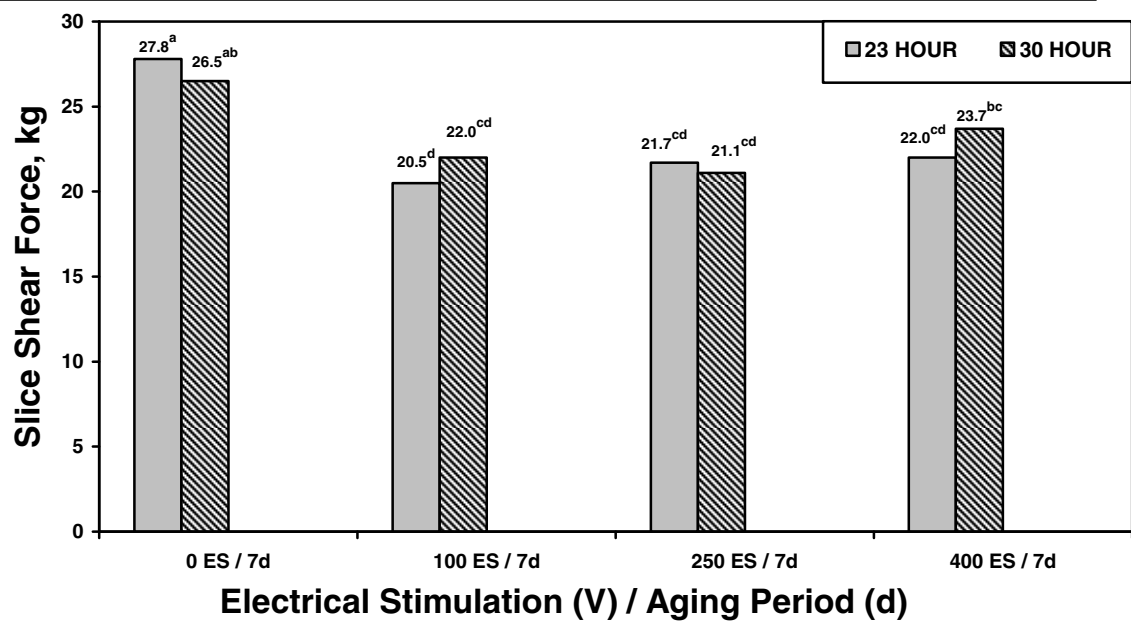


Figure 7. Effect of chilling time and postmortem aging (14 d) on slice shear force values (kg) of electrically stimulated beef carcasses.

^{a,b,c,d} Means lacking common superscripts differ ($P < 0.05$)

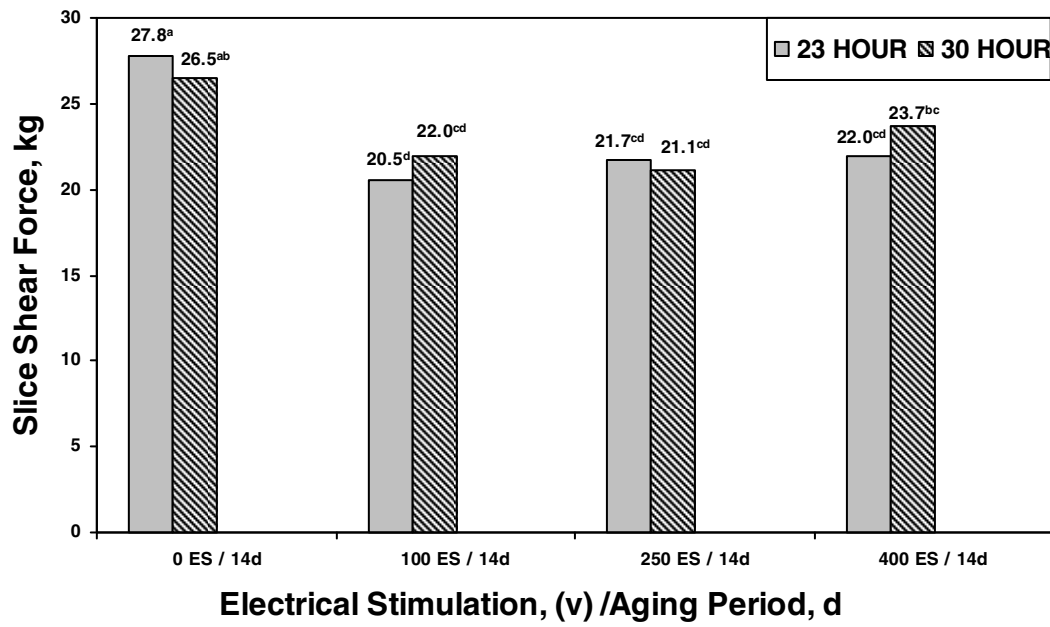


Figure 8. Comparison of percentage of tough vs. tender steaks from different chill times and aging periods of non-electrically stimulated (0 V) beef carcasses
^{a, b}Percentages lacking a common superscript within treatments differ ($P < 0.05$).

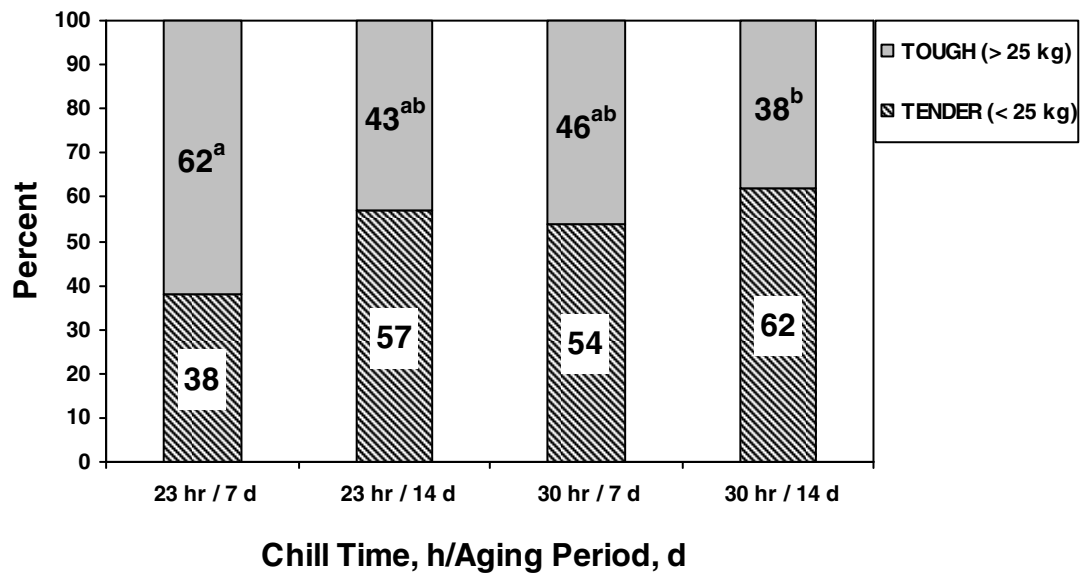


Figure 9. Comparison of percentage of tough vs. tender steaks from different chill times and aging periods of electrically stimulated (100 V) beef carcasses
^{a, b}Percentages lacking a common superscript within treatments differ ($P < 0.05$).

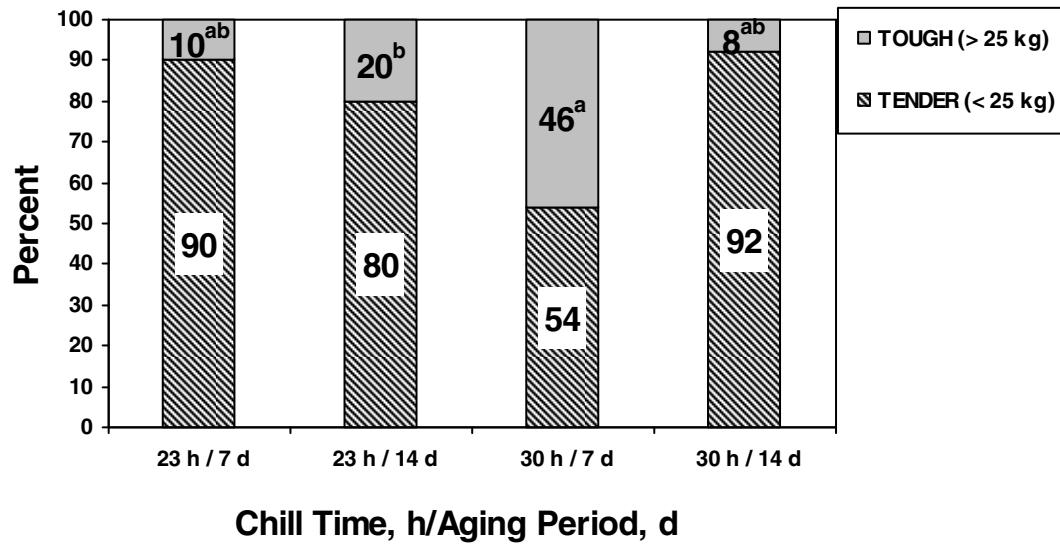


Figure 10. Comparison of percentage of tough vs. tender steaks from different chill times and aging periods of electrically stimulated (250 V) beef carcasses
^{a, b}Percentages lacking a common superscript within treatments differ ($P < 0.05$).

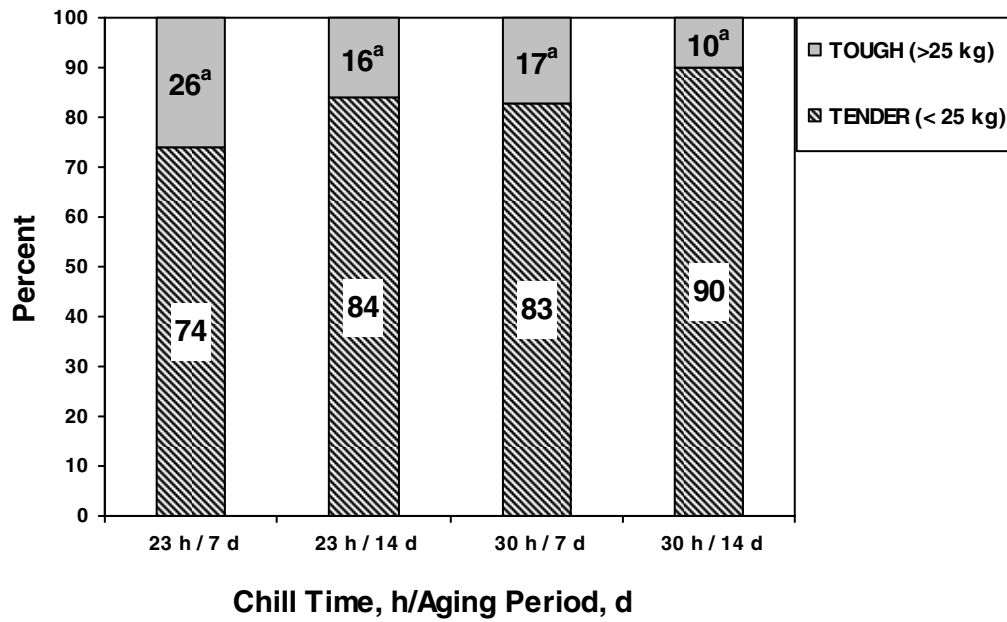


Figure 11. Comparison of percentage of tough vs. tender steaks from different chill times and aging periods of electrically stimulated (400 V) beef carcasses
^{a, b}Percentages lacking a common superscript within treatments differ ($P < 0.05$).

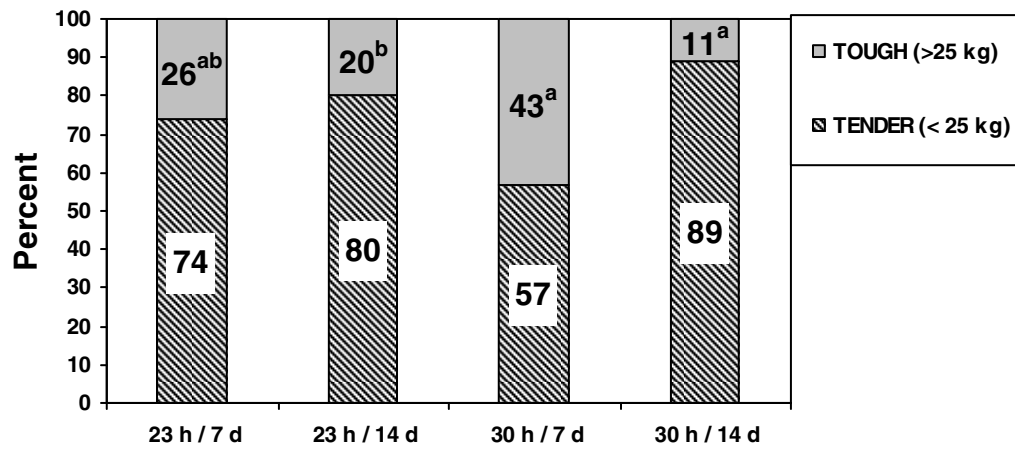
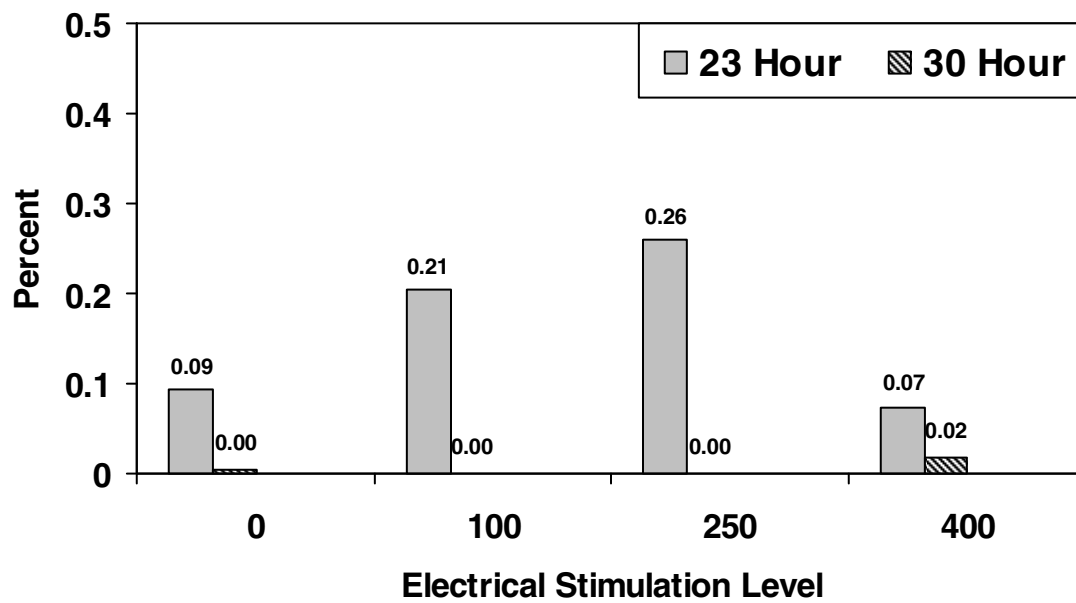


Figure 12. Influence of chill time on shrink loss of electrically stimulated beef carcasses. No difference ($P > 0.05$) in shrink loss existed between chill times or ES levels



CHAPTER IV

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VITA

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Candidate for the Degree of

Master of Science

Thesis: POSTMORTEM INTERACTIONS OF MUSCLE TEMPERATURE,
ELECTRICAL STIMULATION AND MUSCLE PH ON BEEF QUALITY

Major Field: Animal Science

Biographical:

Persoal Data: Born in Shattuck, Oklahoma, on October 18, 1981, the son of
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Date of Degree: May, 2007

Institution: Oklahoma State University

Location: Stillwater, Oklahoma

Title of Study: POSTMORTEM INTERACTIONS OF CHILL TIME AND
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AND OTHER BEEF QUALITY FACTORS.

Pages in Study: 50

Candidate for the Degree of Master of Science

Major Field: Animal Science

Scope and Method of Study: Cattle (n=200) were selected based on common breed type, weight, and fat thickness. Carcasses sides were randomly assigned to one of eight (ES) x postmortem chilling time (h) combinations: 1) 0V/23h; 2) 0V/30h; 3) 100V/23h; 4) 100V/30h; 5) 250V/23h; 6) 250VES/30h; 7) 400VES/23h; 8) 400VES/30h.

Findings and Conclusions: Marbling score did not differ ($P>0.05$) between chilling time or ES levels. Mean values for CIE L^* and a^* of were higher ($P>0.05$) for the 30h chilled carcasses as compared to carcasses chilled 23 h in the non-stimulated group. No color differences ($P>0.05$) were found between ES levels. Slice shear force values were higher for non-stimulated controls at 7 and 14d than for ES sides. Shortening of chill time had no detrimental effects on quality. Electrical stimulation increased tenderness and improved lean color. This evidence could aid the beef industry in increasing throughput and possibly increasing profit.

ADVISER'S APPROVAL: Dr. J. Brad Morgan
