INFLUENCE OF CARCASS VASCULAR RINSING WITH CALCIUM CHLORIDE ON NON-ELECTRICALLY STIMULATED AND STIMULATED CARCASSES ON MEAT QUALITY OF LAMBS

by

Adam J. Franzen

A thesis submitted in partial fulfillment of

the requirements for the degree of

Master of Science

(Animal Sciences)

at the

UNIVERSITY OF WISCONSIN-MADISON

2024

Date of final oral examination: 12/4/2024

The thesis is approved by the following members of the Final Examination Committee:
James R. Claus, Professor and Major Advisor, Animal and Dairy Sciences
Wei Guo, Assistant Professor, Animal and Dairy Sciences
Robert E. Campbell, Ph.D., Director of Technical Services, MPSC Inc.

Abstract of Thesis

INFLUENCE OF CARCASS VASCULAR RINSING WITH CALCIUM CHLORIDE ON NON-ELECTRICALLY STIMULATED AND STIMULATED CARCASSES ON MEAT QUALITY OF LAMBS

Infusing calcium chloride into carcasses before rigor mortis presents a promising avenue for enhancing meat quality and tenderness. This technique targets the activation of μ-calpain and m-calpain, two key enzymes responsible for the postmortem breakdown of myofibrillar and cytoskeletal proteins (Huff Lonergan et al., 2010). The effectiveness of intra-arterial infusion of lambs with a 0.3 M solution of $CaCl_2$ (10% live weight) was previously demonstrated, resulting in reduced shear force across varying postmortem intervals 1, 7, 14 days (Koohmaraie et al., 1991). The emergence of Rinse & Chill® technology (MPSC Inc., Hudson, United States) has further facilitated the commercial viability of calcium chloride infusion into carcasses, fostering opportunities for continued exploration and application of such methods (Hwang et al., 2022). This study aimed to assess the impact of calcium chloride infusion into lamb carcasses on pH decline, meat color and Warner-Bratzler shear force (WBS). It was hypothesized that the infusion of calcium chloride would lead to a reduction in shear force, thereby contributing to increased meat tenderness.

The study was conducted over three different trial periods with lambs (n=40) which consisted of various breeds (commercial crossbred, Dorset, Hampshire, Suffolk, Polypay, Southdown), age (6-9 months), and live body weight (59.4 ±29.5 kg). Lambs were randomly

assigned to three vascular rinse treatments (TRT) that included: (RC= Rinse & Chill® solution; saccharides, phosphates), (CA= 0.3M $CaCl_2$ + RC), (ES-CA= electrical stimulation, 800mA current, peak 350V for two consecutive 30-second applications, followed by CA). Animals were stunned by penetrating captive bolt. The vascular rinsing process entailed inserting a catheter into the heart and rinsing the carcass at 10% of its body weight. Treatments were applied to the carcass immediately upon exsanguination. Carcasses were skinned, eviscerated, and chilled (3°C, 24 h). Carcass temperature and pH were recorded (semimembranosus, SM) from 1 to 20 h postmortem (PM). At 24 h PM, the longissimus dorsi (LD), SM, and triceps brachii (TB) were excised, vacuum packaged, or overwrapped in oxygen-permeable film. Color measurements (CIE L^*, CIE a^*, chemical states of myoglobin) were determined during storage (3 and 7 d PM). Purge, (WBS) on cooked (68.3 °C internal) LD, SM, and TB chops, and cook loss (3 and 7 d PM) were determined. Concentrations of calcium and phosphorus in the muscles were analyzed. Data was analyzed as a split-split plot design with means ($P<0.05$) separated using PROC MIXED (SAS Institute).

Live animal weights were not different ($P>0.05$) among the treatments. Hot carcass weights were not different ($P>0.05$) among the treatments. For carcass pH, the ES-CA treatment was lower ($P<0.05$) than the Ca and RC treatments, while CA was lower ($P<0.05$) than the RC treatment. The RC treatment had greater CIE L^*, CIE a^*, Chroma C, and oxymyoglobin, and lower values for metmyoglobin ($P<0.05$). The SM muscle had lower CIE L^* ($P<0.05$) than the LD and TB muscles. At day 3 PM, CIE a^* was lower ($P<0.05$) in the LD muscle compared to TB and SM. On day 7 PM, CIE a^* was higher ($P<0.05$) in the TB muscle compared to LD and SM. RC had the least ($P<0.05$) purge in the LD and SM muscles. RC had lower ($P<0.05$) cook loss than ES-CA. The LD in CA and ES-CA had lower ($P<0.05$) WBS than RC. With ES-CA, the SM

exhibited the lowest ($P<0.05$) WBS. RC had the greatest WBS at day 3 ($P<0.05$), with no differences ($P>0.05$) found among TRT on day 7. In the LD muscle, calcium had a lower ($P<0.05$) concentration in the RC treatment than CA and ES-CA. In the TB muscle, there was a lower ($P<0.05$) concentration of calcium in RC than in ES-Ca. In the SM muscle, calcium had a lower ($P<0.05$) concentration in the RC treatment than Ca and ES-Ca. No treatment differences were found in phosphorous concentrations. The SM muscle had greater ($P<0.05$) phosphorous concentrations than the LD and TB. The LD had higher ($P<0.05$) phosphorous concentrations than the TB muscle.

The implications of vascularly delivering calcium chloride throughout the carcass on meat quality are noteworthy, particularly shear force reduction, suggesting the potential to enhance tenderness. More rapid chilling to reduce the effect of the rapid drop in pH may benefit meat color and moisture retention.

Keywords: Calcium Chloride, Carcass Vascular Rinsing, Electrical Stimulation, Lamb, Meat Quality

Adam Jacob Franzen

Department of Animal and Dairy Science

University of Wisconsin-Madison

Madison, WI 53706

Acknowledgments

Foremost, I would like to express my deepest gratitude to my mentor, Dr. James Claus. Without his exceptional guidance and unwavering support over the past several years, this thesis would not have been possible. His mentorship created a positive research environment in which I thrived, and his patience and expertise were invaluable in shaping my academic journey. Dr. Claus has provided me with the necessary experience to excel in the meat industry and make substantial contributions. I would also like to extend my heartfelt appreciation to my committee members, Dr. Bob Campbell and Dr. Wei Guo. Their invaluable advice and guidance throughout this process have been instrumental to the completion of this thesis. Their diverse areas of expertise greatly enriched my educational experience.

I extend my sincere appreciation to MPSC, Inc. for their generous funding, which made this project and my master's degree possible. Their support has transformed my academic aspirations into reality, and I am deeply grateful for their contribution. Special thanks are due to Dillon Walker, MSABD's meat lab manager, and his team, for their assistance with the harvest which was essential to this project. Furthermore, I would like to acknowledge the outstanding mentorship of Dr. Koeun Hwang, whose expertise and guidance were instrumental in shaping many aspects of this thesis. I am truly grateful for the methodologies I learned from her, which greatly contributed to the successful outcome of my research.

Finally, as I reflect on this journey, I am filled with profound gratitude for the friendships forged and memories created along the way. Though this chapter of my life is closing, the lessons learned and relationships formed will remain cherished forever. None of this would have been possible without the support and encouragement of these remarkable individuals.

Table of Contents

List of Figures and Tables

Figures:

Tables:

Chapter 1

Introduction

Introducing calcium chloride into carcasses before rigor mortis onset holds promise for enhancing meat quality and tenderness. This approach aims to activate crucial enzymes, μ-calpain and m-calpain, which play key roles in breaking down myofibrillar and cytoskeletal proteins postmortem (Huff Lonergan et al., 2010).

Activation of calpains depends on the presence of calcium, yet sometimes, intramuscular calcium levels may be insufficient for full activation. The longstanding practice of using calcium chloride as a meat tenderizer stems from its ability to elevate intramuscular calcium ions, thereby accelerating the enzymatic breakdown of essential myofibrillar proteins. This process disrupts muscle fiber structure, ultimately enhancing tenderness. Prior studies have shown the effectiveness of infusing lambs with a 0.3 M solution of $CaCl_2$ (10% live weight) via intra-arterial infusion, resulting in reduced shear force at various postmortem intervals 1, 7, and 14 days (Koohmaraie et al., 1991).

It is worth noting that not all studies have consistently shown an improvement in meat tenderness with calcium chloride infusion. For instance, in cattle vascularly infused with 0.3M $CaCl_2$ at 10% live weight using Rinse & Chill® (MPSC Inc), Warner-Bratzler shear force values for the *Longissimus lumborum* were found to be higher than other treatments (Dikeman et al., 2003). It was proposed that incorporating carcass electrical stimulation prior to calcium chloride infusion could deplete readily available ATP and exhaust the muscular system, thereby preventing extensive contraction and toughening (Koohmaraie et al., 1989). Carcass electrical stimulation entails applying electrical impulses to muscles and is renowned for expediting postmortem pH decline by triggering skeletal muscle contractions. Research has consistently

demonstrated its positive impact on meat quality across various meat types, including turkey, lamb, beef, and veal (Aberle et al., 2012).

The emergence of Rinse & Chill® technology (MPSC Inc., Hudson, United States) has furthered the commercial viability of calcium chloride infusion into carcasses, providing avenues for continued exploration and application of such methods. Rinse & Chill® technology, widely adopted in lamb and beef packing plants, involves circulating a chilled solution through the vasculature during exsanguination to expel warm residual blood, thus accelerating carcass chilling and improving yields. Comprising mostly water (98.5%), with additional dextrose, maltose, and phosphates, the isotonic solution has been studied for its effects on various red meat species, including beef, lamb, bison, and recently, pork.

Previous studies on Rinse & Chill® technology in lamb and bison have shown improvements in tenderness and red meat color (Mickelson & Claus, 2020; Fowler et al., 2017). Kethavath et al. (2021) explored its effects on electrically stunned market hogs, suggesting quality enhancements even with rapid pH decline, particularly in suboptimal harvest facility temperature conditions. This study aimed to expand upon previous findings by examining how electrical stimulation and the incorporation of calcium chloride into the rinse solution affect lamb carcasses.

The purpose of this thesis was three-fold:

- To determine the level of delivery of calcium to various muscles within the carcass.
- To assess the impact of calcium infusion on various indices (pH decline, WBS, meat color).

- To evaluate the physical and chemical effects of electrically stimulating the carcass before vascular rinsing with a solution containing calcium chloride.

It was hypothesized that administering electrical stimulation prior to calcium chloride infusion would mitigate extensive toughening, increase rinse solution distribution, and the presence of calcium will lead to decreased shear force and enhanced meat tenderness. This thesis comprises chapters, with Chapter 2 offering a comprehensive review of relevant literature and background knowledge, while Chapter 3 presents the research findings in a format suitable for journal publication.

1.1 References

Aberle, E. D., Forrest, J. C., Gerrard, D. E., & Mills, E. W. (2012). *Principles of Meat Science* (5th ed.). Kendall Hunt Publishing Company. Dubuque IA.

Dikeman, M. E., Hunt, M. C., Addis, P. B., Schoenbeck, H. J., Pullen, M., Katsanidis, E., & Yancey, E. J. (2003). Effects of postexsanguination vascular infusion of cattle with a solution of saccharides, sodium chloride, and phosphates or with calcium chloride on quality and sensory traits of steaks and ground beef. *Journal of Animal Science*, *81*(1), 156–166. https://doi.org/10.2527/2003.811156x

Fowler, S. M., Claus, J. M., & Hopkins, D. L. (2017). The effect of applying a rinse and chill procedure to lamb carcasses immediately post-death on meat quality? *Meat Science*, 134, 124–127. https://doi.org/10.1016/j.meatsci.2017.07.015

Huff Lonergan, E., Zhang, W., & Lonergan, S. M. (2010). Biochemistry of postmortem muscle - lessons on mechanisms of meat tenderization. *Meat Science*, *86*(1), 184–195. https://doi.org/10.1016/j.meatsci.2010.05.004

Hwang, K., Claus, J. R., Jeong, J. Y., Hwang, Y.-H., & Joo, S.-T. (2022). Vascular rinsing and chilling carcasses improves meat quality and food safety: A Review. *Journal of Animal Science and Technology*, *64*(3), 397–408. https://doi.org/10.5187/jast.2022.e29

Kethavath, S. C., Hwang, K., Mickelson, M. A., Campbell, R. E., Richards, M. P., & Claus, J. R. (2021). Vascular infusion with concurrent vascular rinsing on color, tenderness, and lipid oxidation of Hog Meat. *Meat Science*, *174*, 108409. https://doi.org/10.1016/j.meatsci.2020.108409

Koohmaraie, M., Crouse, J. D., & Mersmann, H. J. (1989). Acceleration of postmortem tenderization in ovine carcasses through infusion of calcium chloride: Effect of

concentration and ionic strength. *Journal of Animal Science*, *67*(4), 934–942. https://doi.org/10.2527/jas1989.674934x

Koohmaraie M, Shackelford S.D. (1991) Effect of calcium chloride infusion on the tenderness of lambs fed a beta-adrenergic agonist. *Journal of Animal Science*, 9(6):2463-71. doi: 10.2527/1991.6962463x. PMID: 1679427.

Mickelson, M. A. & Claus, J. R. (2020). Carcass chilling method effects on color and tenderness of bison meat. *Meat Science*, *161*, 108002. https://doi.org/10.1016/j.meatsci.2019.108002

Chapter 2

Literature Review

2.1. Muscle Physiology

2.1.1 Skeletal Muscle Cytology

The individual cell of skeletal muscle is known as a muscle fiber, making up approximately 75 to 92 percent of the total volume of the muscle. Other structures within skeletal muscle include motor nerves, blood vessels, connective tissue, and extracellular fluid. These muscle fibers are unbranched, multinucleated cells that can be several centimeters long. The membrane of the muscle fiber is known as the sarcolemma, and it possesses elastic properties, allowing it to stretch and contract. Along the length of the muscle fiber, there are frequent invaginations of the sarcolemma from a system of tubules called transverse tubules (T-tubules) (Aberle et al., 2012).

The sarcoplasm is the cytoplasm of muscle cells. This substance contains all the organelles of the muscle cell, as well as glycogen granules, lipid droplets, various proteins, and other constituents. Muscle fibers are multinucleated, and the deposition of nuclei is just beneath the sarcolemma in mature cells. Myofibrils are long, thin, rod-shaped organelles that run along the length of the fiber. Each skeletal muscle cell typically contains thousands of myofibrils, which are interconnected and within the sarcoplasm that surrounds them (Aberle et al., 2012)

Within each myofibril, two types of myofilaments, thick and thin, are highly organized and aligned precisely. The overlap of the thick and thin filaments gives the muscle fiber its striated appearance. The sarcomere is the repeating structural unit of muscular contraction and relaxation, consisting of both an A band and the two half I bands located on either side of the A

band. Sarcomere length can vary between different fibers. The myofibril's most abundant proteins include myosin, actin, titin, tropomyosin, troponin, and nebulin. Myosin and actin are considered the major contractile proteins, while troponin and tropomyosin act as regulatory proteins. Titin and nebulin function as cytoskeletal proteins (Aberle et al., 2012). Myosin serves as the primary protein in thick filaments, which constitute the A band of the sarcomere and are anchored in place by several proteins situated at the M line. On the other hand, Actin is the functional protein in thin filaments, forming the I band of the sarcomere while extending partially into the A band. In addition, the ultra-thin filaments, known as Z filaments, make up the Z disc, and their role involves binding to the thin filaments in neighboring sarcomeres (Aberle et al., 2012).

2.1.2 Vascular System Functionality

Exsanguination, also known as bleeding out, refers to the process of bleeding to death. In ritual slaughter, animals are typically slaughtered without prior stunning, and occlusion may occur when the carotid arteries are severed transversely. The carotid arteries, being elastic, quickly retract into their connective tissue coat after the cut, causing the connective tissue to fill with blood and form clots that seal the cut ends. As the heart continues to beat, blood pressure is maintained in the anterior aorta and vertebral artery. The vertebral artery, which supplies blood to the brain, is not severed during the neck cut (FAWC. 2003).

This occlusion phenomenon can delay the onset of insensibility, potentially prolonging the time during which slaughtered animals may experience pain and distress. Studies have shown that in ritual slaughter, sheep generally become insensible within 5-7 seconds, goats within 3-7 seconds, adult cattle within 22-40 seconds, and calves within 10-120 seconds after the neck cut.

These values do not take into account the potential effects of occlusion and its potential to extend the period of sensibility (FAWC. 2003).

One example of increased blood pressure effects is ecchymosis or blood splash. Preslaughter excitement and psychological or emotional stress can lead to an increase in blood pressure in larger blood vessels and an increase in blood volume in smaller vessels. During stunning, there is a further increase in blood pressure and heart rate. The rupture of distended smaller blood vessels may occur due to vigorous muscular contractions during electrical stunning and subsequent struggling. However, if prompt severance of the aorta is achieved after stunning, the blood pressure is rapidly released, reducing the incidence of blood splash. This could be due to a quick drop in blood pressure or prevention of initially reaching high levels (AMPC. 2006).

2.1.3 Skeletal Muscle Contraction

Skeletal muscle fibers can be stimulated to contract by various means. Contractions in skeletal muscle are typically initiated by an electrical stimulus, or action potential, that starts in the brain or spinal cord and travels via a motor nerve to the sarcolemma of the muscle fiber (Aberle et al., 2012). Motor nerves are nerve fibers that transmit stimuli to skeletal muscles. These motor nerves, part of the peripheral nervous system, are primarily made up of neurons. The neuron consists of a long cylindrical structure called the axon and a polyhedral cell body. Attached to the cell body are several branching structures called dendrites. On the motor nerve, Schwann cells wrap around the axon at regular intervals. These Schwann cells are responsible for the secretion of a myelin sheath, which envelops the motor nerve. The myelin sheath insulates the axon against ion movements and increases the transmission speed of electrical impulses (Aberle et al., 2012).

The action potential is transferred from the motor nerve to the skeletal muscle at the myoneural junction on the muscle fiber. The motor nerve endings (axon terminals) form synaptic connections with the muscle fiber at the motor end plate. These synaptic connections involve the release of the neurotransmitter acetylcholine into the synaptic cleft. Acetylcholine binds to nicotinic receptors on the sarcolemma, leading to local depolarization and the propagation of an action potential across the muscle fiber (Guo & Greaser, 2022). There is typically only one myoneural junction for each muscle fiber. Acetylcholine does not merely amplify the signal but is essential for transmitting the signal from the motor neuron to the muscle fiber. The local depolarization caused by acetylcholine binding triggers the opening of voltage-gated sodium channels, initiating an action potential that travels along the sarcolemma and into the T-tubules (Guo & Greaser, 2022).

The effects of the action potential on the muscle fiber last from 5 to 10 milliseconds. Depolarization of the T-tubules is sensed by calcium release channels in the sarcoplasmic reticulum (SR), known as ryanodine receptors, leading to the release of calcium ions into the sarcoplasm. Calcium ions then bind to troponin, initiating the contraction cycle (Guo & Greaser, 2022). The enzyme cholinesterase is released by the motor end plate to terminate the action potential, ensuring that acetylcholine is degraded and the signal does not persist indefinitely.

With muscle in the relaxed state, calcium is sequestered in the terminal cisternae of the SR, and the concentration of calcium in the cytoplasm is low (10^{-7} moles/liter). Meanwhile, there are high concentrations of ATP (adenosine triphosphate) complexed with magnesium (Mg^{2+}) ions forming the Mg-ATP complex. This Mg-ATP complex and low sarcoplasmic calcium ion concentrations prevent the interaction of actin and myosin (cross-bridge formation). Calcium

concentrations need to increase to about 10^{-6} or 10^{-5} moles/liter to initiate a contraction (Aberle et al., 2012).

Troponin is a regulatory protein, composed of three subunits, responsible for controlling cross-bridge formation. These subunits are designated troponin T, troponin I, and troponin C. When calcium ions are released from the terminal cisternae of the SR, they bind to troponin C, causing a conformational change in the entire troponin complex. This movement shifts tropomyosin off the myosin binding sites on actin, allowing cross-bridge formation between actin and myosin (Guo & Greaser, 2022). The protein complex of the cross-bridge formation is known as actomyosin. The length of the thick and thin filaments does not change during contraction. Instead, the filaments slide along each other, pulling the Z-disks closer together, resulting in a decrease in sarcomere length (Aberle et al., 2012).

Skeletal muscle contraction requires energy, which is provided by ATP. During cross-bridge formation, the myosin head hydrolyzes ATP into ADP (adenosine diphosphate) and inorganic phosphate, releasing the energy needed for muscle contraction. The sliding of actin and myosin filaments is driven by the energy released from ATP hydrolysis (Guo & Greaser, 2022). After the sarcomere shortens, calcium ions are returned to the SR by means of calcium sequestering. For calcium ions to be returned to the terminal cisternae of the SR, they must go against a concentration gradient. This process requires ATP and is mediated by the calcium pump, or SERCA (sarcoplasmic/endoplasmic reticulum calcium ATPase, Guo & Greaser, 2022). Once calcium is no longer bound to troponin C, tropomyosin moves back into its blocking position on actin, and the filaments slide back to their original position.

2.1.4 Postmortem Muscle Metabolism

The conversion of muscle to meat begins with exsanguination which destroys the circulatory system and disrupts homeostasis (Aberle et al., 2012). Skeletal muscle will attempt to maintain homeostasis, by buffering against a loss of ATP for as long as possible. The three ways skeletal muscle can generate ATP are the phosphagen system, glycolysis, and mitochondrial respiration. With the phosphagen system, cellular ATP is maintained by creatine being phosphorylated with ADP. When ATP levels drop below a certain level, phosphocreatine is the first source to resynthesize ATP (Aberle et al., 2012).

$$H^+ + ADP + phosphocreatine \rightleftarrows ATP + creatine + H_2O$$

The catalyst for this reaction in both directions is creatine kinase. Muscle cells will generate ATP from ADP using the enzymes adenylate kinase, or myokinase during times of sustained contraction. A byproduct of ATP synthesis using adenylate kinase or myokinase is adenosine monophosphate (AMP). The presence of AMP in muscle cells is a biochemical signal of low ATP. AMP is quickly deaminated postmortem to try and maintain ATP production (Aberle et al., 2012).

Once the phosphagen system is incapable of replenishing ATP, the muscle will use glycolysis, the breakdown of glucose through anaerobic metabolism, to regenerate 2 net ATP. The production of ATP from glycolysis occurs primarily in the sarcoplasm of the muscle. Glycogen is the stored form of glucose in the muscle and makes up about 1 percent of the total muscle weight. Since the circulatory system is not functional postmortem, anaerobic metabolism is the only way to replenish ATP. The process is accomplished by converting pyruvate, the final product of glycolysis, into lactate (Aberle et al., 2012).

$$Pyruvate + NADH + H^+ \rightarrow Lactate + NAD^+ + H^+$$

In anaerobic conditions, lactate accumulates in the muscle because pyruvate is converted into lactate to regenerate nicotinamide adenine dinucleotide (NAD+). NAD+ is an important product for several reactions upstream in the glycolytic pathway to regenerate ATP. The concentration in H+ increases in the muscle because of lactic acid production. Each time ATP is hydrolyzed, 1H+ is released into the cell. 1H+ is also released with each glucose residue from glycogen. This increase in H+ quickly exceeds the buffering capacity of the muscle cell and the pH of the tissue declines (Aberle et al., 2012). The pH continues to decline until there is no more available ATP, and ADP cannot be rephosphorylated. The ultimate pH in goat carcasses typically ranges from 5.8 to 6.2 (Webb. 2014). Muscle remains extensible until there is a lack of ATP to break the actomyosin bonds. This permanent formation of cross-bridges, and inextensibility of muscle, is known as rigor mortis (Aberle et al., 2012).

During the postmortem period, biochemical activities occur that improve meat tenderness. After rigor mortis, skeletal muscle will regain some extensibility as proteolytic degradation of specific myofibrillar proteins results in dissolution of the Z discs. The proteins desmin, nebulin, and titin are the predominant Z disk proteins that are degraded through enzymatic activity. Several enzyme systems responsible for postmortem degradation of myofibrillar proteins include calpains, the multi-catalytic proteinase complex, and cathepsins. When the Z disks degrade, it causes myofibrils to rupture at the Z disk-I band junction. This releases tension from cross-bridge formation to make the muscle more extensible (Aberle et al., 2012).

2.2 Calpain Activity

Meat tenderness is an essential characteristic that significantly shapes the dining experience and overall satisfaction of consumers. Over several decades, spanning from the 1920s to the 1970s, research established the foundational knowledge to grasp how genetics, biochemistry, and production factors influence meat tenderness. Key determinants of tenderness encompass elements like connective tissue, myofibrillar integrity, sarcomere length, and intramuscular fat. Recent advancements have further deepened the comprehension of molecular aspects such as glycolysis, calcium release, and protease activation. Taking into account the impact of these factors on tenderness across diverse animal variations, the enzymatic processes during postmortem aging emerge as a pivotal mechanism for enhancing meat tenderness (Warner et al., 2022).

Enzymatic activities play a crucial role in breaking down proteins and other components of muscle fibers during postmortem aging. One such group of enzymes that are of substantial importance during this process is a class of calcium-dependent proteases. These calpain enzymes are naturally present in muscle tissues but remain inactive until triggered by an increase in calcium ions (Ca^{2+}), which occurs during postmortem aging. Activation of calpains occurs through calcium binding to the regulatory domain, triggering a conformational change that exposes the active site of the enzyme. Calpains function by cleaving peptide bonds within muscle proteins, including myofibrillar proteins such as myosin and actin, as well as cytoskeletal and other structural proteins. This enzymatic process leads to the breakdown of muscle fiber structure, disrupting the fibrous network and reducing the force required to chew the meat. The extent of meat tenderization achieved through calpain activity depends on various factors, such

as the duration and conditions of aging, temperature, and pH levels (Huff-Lonergan & Lonergan. 2005).

In living muscle, the concentration of free calcium ions in the sarcoplasm is maintained at a low level through the action of Ca2+ pumps located within the SR and mitochondria. In resting muscle, the free calcium concentration is approximately 0.2-0.8 μM (Koohmaraie et al., 1992). During muscle contraction sarcoplasmic calcium concentrations can increase to over 5 μM (Ertbjerg & Puolanne. 2017). However, in postmortem muscle, the Ca2+ pumps cease to function due to the depletion of ATP. As a result, there is a leakage of Ca2+ from the mitochondria and SR, leading to an elevation in sarcoplasmic calcium concentrations. In the case of lamb carcasses, it has been observed that the free calcium concentrations reach a plateau of 110 μM by the time the ultimate pH of 5.3 is reached (Hopkins & Thompson. 2001). In the USDA's Food Data Central, a 100g sample of Lamb, New Zealand, imported, loin, boneless, separable lean and fat, raw contains 4mg of calcium (USDA. 2019).

The interaction of muscle pH and temperature during rigor is closely tied to meat tenderness, as they collectively influence the extent of physical contraction and the activity of proteolytic enzymes. Following slaughter, the pH of muscle tissue begins to decline due to anaerobic glycolysis, which can enhance calpain activity. Veiseth et al. (2001) demonstrated that postmortem tenderization is a result of μ-calpain-mediated proteolysis of crucial myofibrillar and cytoskeletal proteins. Furthermore, m-calpain is also involved in the breakdown of myofibrillar proteins (Huff Lonergan et al., 2010). Each of these calpain enzymes is triggered by distinct calcium ion concentrations. μ-calpain becomes active when exposed to calcium ion levels ranging from 50 to 70 μM, whereas m-calpain requires calcium ion concentrations of 1 to 5 mM to initiate its activation (Polidori et al., 2000). μ-Calpain exhibits increased activity at a pH of 6.5

when contrasted with pH levels of 7.5 and 6.0. In contrast, m-calpain activity is greater at pH 7.5 than at pH 6.5 (Maddock, 2005). Research conducted by Hwang & Thompson (2001) highlighted that the relationship between meat tenderness μ-calpain activity post-mortem is not always consistent. In their investigation, samples with higher rigor temperatures (38°C at pH 6.0) had an earlier activation of μ-calpain. This resulted in more tender meat at day 1 postmortem. However, with the progression of aging, the tenderness observed was less pronounced compared to samples subjected to lower rigor temperatures. These findings are supported by Dransfeld (1994) who proposed that elevated temperatures can lead to the premature inactivation of enzymes. This creates a situation where proteolysis starts off quickly, but there is minimal extended aging over time. Alterations in pH and ionic concentrations can also induce structural modifications in both enzymes and their substrates, thereby impacting enzyme functionality and the vulnerability of substrates. Carlin et al. (2006), observed the activity of μ- and m-calpain was diminished when exposed to 295 mM NaCl compared to 165 mM NaCl, across pH levels of 7.5, 6.5, and 6.0.

2.3 Cold Shortening

The texture of meat can be severely impacted by the rapid chilling of pre-rigor muscle. Pre-rigor muscles hold ample reserves of the contractile energy source, adenosine triphosphate (ATP), which enables forceful contraction to initiate as the temperature drops below 10°C, with the greatest impact observed around 3°C. Cold shortening refers to the abnormal contraction of pre-rigor skeletal muscle that can occur when it is rapidly cooled, below 10 °C, before the meat pH has fallen below approximately 6.2 (James & James. 2002). Muscle shortening up to 20% only marginally increases toughness, but when shortening falls within the range of 20% to approximately 40%, the toughness increases substantially. Once shortening surpasses 40%, the

meat becomes much more tender. At a 60% reduction, it becomes nearly as easy to cleave meat that has not shortened (Marsh & Leet. 1966). Rapid cooling leads to a decrease in temperature, which affects the biochemical reactions and physical properties of the muscle fibers. When post-mortem muscle is above 12 °C, it remains in a passive, relaxed state until ATP levels start to decline during the rapid phase of rigor. In the context of cold shortening, the calcium-pumping mechanism plays a substantial role (James & James. 2002). In living muscle, calcium pumps actively transport calcium ions from the sarcoplasm back into the SR using energy from ATP hydrolysis. This process reduces sarcoplasmic calcium levels, allowing muscle relaxation after contraction. In postmortem muscle, these pumps stop functioning, leading to calcium ions leaking out of the terminal cisternae of the SR due to concentration gradients.

There is likely some leakage from the calcium storage vesicles when muscle is at rest, requiring the calcium pump to function continuously, albeit at a slow pace, to maintain the low resting level of intracellular Ca^{2+} concentration. Additionally, the calcium pump exhibits a high temperature dependency. At 10 °C, it operates at approximately 1/200th of the rate at the body temperature of around 38 °C, and at 2 °C, it functions at only about 1/1000th of the rate (James & James. 2002). Consequently, as the temperature decreases, especially below 10 °C, there is an increase of Ca^{2+} leakage into the myofilaments. Free calcium ions in the myofilaments and bind to troponin C, which moves tropomyosin away from the myosin-binding sites on actin filaments. This exposure allows cross-bridge formation, leading to muscle contraction as the preloaded myosin head from the hydrolysis of ATP is utilized, resulting in the characteristic contraction observed in cold shortening and intensifying the production of ADP. Subsequently, ADP stimulates the reactions involved in ATP synthesis (James & James. 2002).

Quick cooling offers practical advantages but also raises the risk of cold shortening. The speed of glycogen breakdown into lactic acid varies among different species. In lamb and beef, glycolysis occurs at a slower rate than pork, resulting in a gradual decrease in pH. Consequently, it is relatively easy to cool the carcasses of these animals, especially the outer layers, to temperatures below 10 °C while the pH remains above 6.2. Under these conditions, such carcasses become highly susceptible to cold shortening. Conversely, pork carcasses, due to higher fat insulation, retain heat for a longer time compared to lamb. This, coupled with the quicker onset of rigor mortis, makes cold shortening less likely (James & James 2002).

Cold shortening does not affect the muscles within a carcass uniformly. Previous research showed the *sternomandibularis* was partially shielded from cold, which prevented the localized shortening, except in the section of the muscle directly exposed to the cold air. It was suggested that "cold-shortening" could potentially happen to muscles within the carcass that have lower fat coverage, are not under tension, and are not close to bone (Marsh & Leet. 1966).

2.4 Calcium Chloride

Calpains rely on the presence of calcium for activation, but sometimes, the intramuscular calcium concentration may not be sufficient for full activation. The practice of using calcium chloride ($CaCl_2$) as a meat tenderizer is well-established, mainly because it can activate the calpain proteolytic system during the postmortem aging process. By elevating the intramuscular concentration of calcium ions, calcium chloride accelerates the enzymatic breakdown of crucial myofibrillar proteins, leading to the disruption of muscle fiber structure and an enhancement in tenderness.

In a previous study lambs received an intra-arterial infusion of 0.3M solution of $CaCl_2$ at 10% live weight. The results showed a decrease in shear force within the *longissimus* muscle at all postmortem times (days 1, 7, 14). The calcium chloride infusion also increased marbling, improved lean color score, increased dressing percentage and decreased lean firmness (Koohmaraie et al., 1991).

Infusing lamb carcasses with $CaCl_2$ at a concentration of 0.3M was better at improving tenderness when compared to infusion concentrations of 0.075M or 0.15M (Koohmaraie et al., 1989). The need for such elevated levels of $CaCl_2$ might be due to the obstacles hindering the movement of ions into the cell. A high concentration of $CaCl_2$ ensures that some of these ions effectively reach their intended enzymes by overwhelming the cells. For this application, the effective concentration of Ca^{2+} is substantially higher than what would naturally occur because the maximum Ca^{2+} concentration is not achieved until the postrigor period.

Injecting 0.175 M - 0.25M $CaCl_2$ solutions at 5% - 10% of the meat weight into beef, 30 minutes or 24 hours postmortem, lowered shear force values in the *longissimus*, *semimembranosus*, and *triceps brachii* (Wheeler et al., 1993). Beef top loin and inside round subprimals were removed from the carcass and injected with 0.2M $CaCl_2$ at 5% meat weight at 30 hours postmortem. The samples were then aged to 7 days postmortem. Trained sensory panels showed improved tenderness with $CaCl_2$ injection in both *Longissimus Dorsi* and *Semimembranosus* muscles, while not causing off-flavor problems or affecting flavor intensity, when compared with controls (Lansdell et al.,1995).

Not all studies have demonstrated an improvement in meat tenderness with calcium chloride infusion. When cattle were vascularly infused by Rinse & Chill® (MPSC Inc), with 0.3M $CaCl_2$ at 10% live weight, the Warner-Bratzler shear force values for the L*ongissimus*

lumborum were higher than other treatments. Using a descriptive attribute and sensory panel in the same study, the L*ongissimus lumborum* was found to be less tender than other treatments. The failure of calcium chloride to improve tenderness may be the result of Ca^{2+} induced contraction. With readily available ATP in the muscle, an infusion of calcium chloride would be able to trigger extensive contraction and subsequent toughening (Dikeman et al., 2003). It was proposed that implementing carcass electrical stimulation prior to an infusion of calcium chloride would deplete readily available ATP, and prevent extensive contraction and toughening (Koohmaraie et al., 1989).

2.5 Postmortem Technologies

2.5.1 Infusion

Infusion refers to the process of introducing a substance or ingredient into veins, arteries or tissues. There are different types of infusion procedures, such as transport infusion and injection infusion. Injection aims to deliver a solution containing ingredients or substrates into the tissue, with the intention of the tissue retaining the solution. Vasculature infusion, on the other hand, involves the passage of the solution through the vasculature and is designed to allow the solution to exit the body.

In the context of meat processing, injection is a commonly used method to deliver a solution to specific cuts of meat using different techniques such as artery pumping, stitch pumping, and multiple stitch injection. The goal is to distribute the solution quickly and uniformly throughout the tissue. Artery pumping involves delivering the solution through the vascular system under controlled pressure to ensure successful delivery. Stitch pumping entails injecting the solution multiple times into the muscle cut, particularly in the thickest area, using a

hollow needle. Multiple stitch injection is a variation of stitch pumping that employs a series of hollow needles to deliver the solution simultaneously (Aberle et al., 2012). These methods are typically used to make whole muscle, cured and processed meats such as bacon, and bone-in hams.

The effect of calcium chloride injection on the tenderness, aging rate, and meat quality of pork *longissimus* muscle was investigated. The experiment involved two different infusion times of (0.5 or 6 hours postmortem) using a hand-held brine injector fitted with a three-prong infusion fork. The study found that calcium chloride infusion improved meat tenderness but had adverse effects on drip loss and meat color (Rees et al., 2002).

2.5.2 Vascular Rinsing and Chilling (Rinse & Chill®)

Rinse & Chill® Technology (RCT) is an innovative carcass vascular rinsing method developed by MPSC (Hudson, Wisconsin, United States) to enhance meat quality and safety attributes for various species. This technology takes advantage of the circulatory system in the post-exsanguination carcass to improve various meat quality aspects. The process involves the insertion of a specially designed catheter into the carotid artery or heart immediately after exsanguination. Through this catheter, a chilled solution containing various phosphates and saccharides is delivered to the cardiovascular system, enabling the removal of residual blood, accelerated carcass cooling, and manipulating pH decline. The amount of rinse solution applied to an individual carcass can reach up to 10% of the carcass weight (Hwang et al., 2022). The duration of the Rinse & Chill® application varies depending on the livestock species. For lamb carcasses, the process typically lasts about 15 seconds, while for beef carcasses, it lasts approximately 3-4 minutes. After the completion of the vascular rinsing process, the catheter is

removed, and the carcass proceeds through the traditional harvest procedures (Hwang et al., 2022)

The rate of glycolysis and subsequent pH decline can be manipulated through the pre-rigor infusion of the carcass. (Farouk and Price. 1994) conducted a lamb study and found that glycolysis was completed in 6 hours when the carcass was rinsed, while it took 12-24 hours for the non-rinsed carcass. More recent studies, such as Kethavath et al. (2021), have shown that carcasses infused with the MPSC solution exhibited faster pH declines within 24 hours postmortem in cull dairy cows and within 4 hours postmortem in market hogs. This accelerated pH decline can be attributed to the specific ingredients present in the isotonic solution, which have an impact on glycolytic enzymes. Phosphates, for instance, are likely responsible for facilitating anaerobic metabolism, while saccharides such as dextrose and maltose serve as a source of glucose that, under anaerobic conditions, leads to lactic acid formation. Therefore, these compounds likely regulate crucial steps in the glycolytic pathway, influencing the rate and overall decline in pH (Kethavath et al., 2021).

Rinse & Chill® Technology (RCT) has been shown to have beneficial effects on meat color. When cull dairy cows were vascularly rinsed (RCT), it produced greater redness, CIE a^*, with blooming and display time compared to control treatments (Kethavath et al., 2022). Generally, the utilization of Rinse & Chill® Technology leads to meat colors that are lighter, with increased CIE L^*, and redder, with elevated CIE a^* values and higher levels of deoxymyoglobin, along with reduced metmyoglobin, in the *triceps brachii*, *longissimus lumborum*, and *semimembranosus*, across different animal species (Hwang. 2022). Rinse & Chill® applied in lamb carcasses also led to lighter CIE L^* values, particularly when combined with post-evisceration electrical stimulation. Although consistent increases in CIE a^* values were not

observed across all muscles, the treatment generally enhanced lightness and could influence myoglobin oxidation states, with vascularly rinsed samples showing more favorable color stability over display periods (Mickelson et al., 2025).

Tenderness has also been shown to improve when Rinse & Chill® Technology is applied to carcasses of various species. In a study conducted by Farouk et al. (1992), cull dairy cows were vascularly rinsed (RCT) at 10% live weight. The results from this study showed a reduction in shear force within the *supraspinatus*, *longissimus*, and *semitendinosus* muscles. In bison, RCT was able to reduce shear force compared controls (Mickelson & Claus. 2020).

Carcass electrical stimulation involves applying electrical impulses to muscles, triggering a depolarization wave that propagates around the cell membrane and t-tubules, releasing calcium and causing muscle contraction through the binding of myosin to actin. This process, governed by ATP availability, enables cross-bridge formation and detachment, resulting in repetitive sarcomere shortening. It is well-known for expediting postmortem pH decline by triggering skeletal muscle contractions, and research has demonstrated its positive impact on tenderness and overall meat quality in various meat types such as turkey, lamb, beef, and veal (Aberle et al., 2012). However, it's important to note that pork does not experience the same benefits from electrical stimulation. Instead, this process can increase the likelihood of producing pale, soft, and exudative (PSE) meat in pork. Early postmortem electrical stimulation has shown to be effective in inducing PSE development in pigs with different muscling and HAL gene status (Bowker et al., 1999).

2.5.3 Electrical Stimulation

One well-known mechanism of electrical stimulation is its ability to hasten the decline in pH after slaughter by inducing muscle contractions, thereby accelerating rigor mortis. Devine (2009) explained that electrical stimulation is characterized by specific pulse patterns and durations, and that it is impossible to overstimulate the meat because once the pH reaches a certain level, further contraction ceases.

Currently, the industry employs three types of electrical stimulation methods: Extra low-voltage electrical stimulation (ELVES), low-voltage electrical stimulation (LVES), and high-voltage electrical stimulation (HVES). ELVES utilizes a voltage below 100 volts, while HVES operates above 110 volts. LVES, also known as medium-voltage electrical stimulation, is commonly employed when there is a short delay of 10 to 20 minutes between bleeding and stimulation. On the other hand, HVES allows for a longer delay of up to 60 minutes after exsanguination, but it necessitates significant financial investment and extensive safety precautions to safeguard workers (Adeyemi and Sazili. 2014).

Hwang and Thompson (2001) examined the effects of different voltage types (low versus high) applied at various postmortem times on the carcasses of pasture-fed cattle. The mean age, live weight and carcass weight of the cattle were 660 days, 444 kg and 270 kg, respectively. Following slaughter, the striploin was taken from each carcass's side. Samples were then vacuum packed, randomly assigned to 1, 3, 7, or 14-day aging periods at 1°C, and then frozen at −20°C for analysis. The tenderness score and shear force values, specifically focusing on the *Longissimus thoracis* muscle, indicated that the type of electrical stimulation applied three minutes postmortem did not significantly affect the meat tenderness. Their results did show that an early application, three minutes postmortem, of electrical stimulation led to a rapid decline in

pH and reduced meat quality. However, when electrical stimulation was applied at 40 minutes postmortem, better results were observed. The authors suggested that these findings could be attributed to the delayed activation of the calpain system due to elevated calpastatin levels in the carcasses that received an early application of electrical stimulation.

Bendall et al. (1976) demonstrated that the bovine response deteriorated when electrical stimulation was applied after 50 minutes postmortem. They recommended that electrical stimulation be carried out no later than 30 minutes after exsanguination for optimal results. According to Aberle et al. (2012), the tenderization effect of electrical stimulation primarily stems from its ability to prevent cold shortening (accelerated onset of rigor), facilitate calcium release, and disrupt fiber structure through intense muscle contraction. The significance of these mechanisms depends on the chilling conditions.

Slaughter plants often prefer to use electrical stimulation due to its positive impact on meat quality characteristics, in addition to facilitating faster bleed-out. Electrical stimulation has shown to improve tenderness and contribute to desirable attributes such as bright red muscle color, increased muscle firmness, reduced occurrence of dark cutters, and accelerated development of marbling compared to unstimulated carcasses (Aberle et al., 2012).

A study by Cetin et al. (2012) found that electrical stimulation treatment led to increased lightness (L^*) and redness (a^*) values, while a notable decrease was observed in yellowness (b^*) of lamb and goat meat compared to controls (no electrical stimulation). Furthermore, electrical stimulation was shown to enhance water activity, improve water holding capacity, and decrease drip loss in lamb and goat meat, in addition to improving color (Cetin et al., 2012; Aberle et al., 2012).

While electrical stimulation has shown its capability to enhance the lightness (L^*) and redness (a^*) attributes of meat, it has limited effectiveness in improving the color of "dark cutting" meat. This type of meat exhibits an unusually dark purplish-red to black appearance in its lean portion and is often referred to as dark, firm, and dry (DFD) meat. The root cause of this quality defect can be attributed to reduced glycogen reserves within the muscle prior to slaughter. Animals subjected to prolonged pre-slaughter stress substantially deplete their glycogen reserves. Rested and non-stressed animals typically have muscle glycogen levels of 0.8% to 1.0%. When glycogen levels drop below approximately 0.6%, it impedes the typical postmortem pH decrease. Unstressed animals typically have a pH range of 5.4-5.7 in their meat, whereas DFD meat registers a notably higher pH ranging from 5.9 to 6.5, with some instances reaching as high as a pH of 6.8 (Miller. 2007). The depletion of muscle glycogen can result from various pre-slaughter stresses, including transportation fatigue, fear, and adverse environmental conditions. Given the low glycogen reserves in the muscle before slaughter, electrical stimulation is typically ineffective in improving color in DFD carcasses.

2.6 Fresh Meat Properties

2.6.1 Fresh Meat Color

Color plays a substantial role in consumers' purchasing decisions when it comes to fresh meat, as it is often regarded as an indicator of freshness and wholesomeness (Page, Wulf, & Schwotzer. 2001; Mancini & Hunt. 2005). A study was carried out involving 93 meat consumers categorized into two groups based on their familiarity with fresh meat products: high and low familiarity. Both groups reported that the most crucial factor influencing their purchase of fresh meat was its appearance, and the level of familiarity did not affect this outcome (Borgogno et al., 2015).

Consumers have certain expectations regarding the color of different types of meat. For example, fresh beef is expected to have a bright cherry red color when displayed in retail cases, while pork is expected to have a grayish pink color. Lamb and mutton are associated with light red and brick red colors, respectively. In fact, approximately 15% of all retail beef is discounted due to color variations (Aberle et al., 2012). To study meat color, quantitative measures such as colorimetry and reflectance spectrophotometry have been employed in the evaluation of meat color. These methods provide objective measurements to assess and analyze the color characteristics of meat samples.

Color in meat can be described in terms of three attributes: hue, chroma, and value. Hue refers to the wavelength of light radiation, while chroma (or purity/saturation) describes the intensity of a fundamental color relative to the amount of white light mixed in it. Value indicates the overall light reflectance or brightness of the color. In colorimetry, specific measurements are taken to determine the lightness (CIE L^*), redness (CIE a^*), and yellowness (CIE b^*) values of an object (AMSA. 2012).

The quantity of myoglobin, the pigment responsible for muscle color, varies based on factors such as species, age, sex, muscle type, and physical activity. This variability contributes significantly to the variation in meat color. Generally, muscles from older animals with higher myoglobin concentrations tend to be darker in color, while those from younger animals with lower myoglobin concentrations appear lighter (Aberle et al., 2012).

Reflectance spectrophotometry is a technique utilized to assess the relative pigment states of muscle. Myoglobin, the primary pigment in muscle, consists of two components: a protein portion known as globin and a non-protein portion called the heme ring. The color of myoglobin is influenced by different forms of the pigment. Oxymyoglobin (OMb) represents the bright

cherry red color typically associated with fresh meat. Deoxymyoglobin (DMb) is the natural color found in the interior of unexposed intact muscle and is often described as a dark purplish-red shade. On the other hand, metmyoglobin (MMb) refers to an undesirable oxidized brown color (Nguyen et al., 2019).

The oxidative state of the iron within the heme ring determines the binding and modification of the pigment's color. For oxygen to bind, the heme iron must be in the reduced state (ferrous, Fe^{2+}). MMb is the only chemical state of myoglobin in which the heme iron is oxidized (ferric iron, Fe^{3+}). If oxygen binding does not occur, the bloom, which is the brightening of meat upon exposure to oxygen, does not take place. Therefore, MMb is unable to bloom (Mancini and Hunt, 2005).

The color of fully bloomed meat is considered the ideal color in relation to consumer preference, and it is characterized by specific L^* (lightness) and a^* (redness) values according to the guidelines provided by the American Meat Science Association (AMSA. 2015). Factors that contribute to an increase in muscle myoglobin concentration tend to decrease the L^* (lightness), b^* (yellowness), and hue values, while increasing the a^* (redness) and chroma values of fully bloomed meat. This is due to the preferential absorption of different wavelengths of light by myoglobin in the muscle (Calnan et al., 2017).

Color measurements can vary, and different formulas and indices have been developed to assess specific aspects of myoglobin states in meat. One commonly used formula is the K/S ratio, which involves the coefficients of absorption (K) and scattering (S) at specific wavelengths. Specifically, the formula K/S 610 / K/S 525 is considered a reliable indicator of OMb concentration (Mancini and Hunt. 2005). This formula calculates the ratio of the absorption and scattering properties at 610 nm and 525 nm, respectively, to estimate the level of OMb.

Similarly, AMSA provides color guidelines that include formulas for estimating the concentrations of DMb, MMb, and OMb. These formulas involve measuring the reflectance values at specific wavelengths. The formula for DMb is (% reflectance at 474 nm / % reflectance at 525 nm), for MMb it is (% reflectance at 572 nm / % reflectance at 525 nm), and for OMb it is (% reflectance at 610 nm / % reflectance at 525 nm) (AMSA, 2015). These formulas and indices help quantify and evaluate the relative concentrations of different myoglobin states in meat, providing insights into color characteristics and changes.

Hue, a fundamental characteristic of color, is commonly quantified by a singular numerical value, often indicating its position along an angular axis within a color space coordinate diagram, typically centered around a neutral point. The hue angle can be calculated using the formula ($\tan^{-1}(\frac{b*}{a*})$). Chroma C is the measure of color saturation and can be described as the intensity or prominence of a specific hue. At the outer periphery of the hue wheel lie highly saturated hues, exhibiting strong chroma. As one moves towards the center of the color wheel, no single hue predominates, and colors gradually lose saturation. Chroma C can be measured using the formula ($\sqrt{(a*)^2 + (b*)^2}$).

2.6.2 Tenderness

A reliable method for assessing tenderness involves measuring the sarcomere lengths within the muscle. Sarcomeres, which are the smallest contractile units and serve as the force-generating components of striated muscle, undergo contraction during the postmortem period and become fixed once ATP is depleted, signifying the onset of rigor mortis. Factors such as cooling rate, mechanical restraint, and pH decline rate influence postmortem sarcomere lengths. The shortening of sarcomere lengths is described as rigor contraction, thaw rigor, cold

shortening, and heat shortening. These changes, in conjunction with the molecular architecture of the sarcomere involving myosin filaments (S-1 and S-2 units) and their interaction with actin filaments, are influenced by the boundaries created by Z-discs. These factors collectively impact fundamental meat quality attributes, including tenderness and water-holding capacity (Ertbjerg and Puolanne. 2017).

Various methods can be employed to quantify sarcomere length. For instance, (Mickelson. 2018) utilized a helium-neon laser to measure sarcomeres, with a distance of 100mm between the specimen and the diffraction pattern screen. Sarcomere length was calculated following the methodology outlined by Cross et al. (1981).

Warner-Bratzler shear force (WBS) is another widely used measure of meat tenderness. WBS testing involves measuring the maximum force (in Newtons, N) required to shear or cut through a sample of meat as a function of knife movement (in millimeters, mm). This measurement provides an indication of the hardness or toughness of the meat. The term "shear" refers to the slicing of meat parallel to the plane of contact, with the applied force acting tangentially to the segment (Novakovic and Tomasevic. 2017).

To perform WBS testing, texture analyzers are utilized with a specific blade or head attachment. It is important to note that variations in blade thickness, angle, distance of force, and clearance exist among different devices, and these variations should be reported accurately to ensure reproducibility of the WBS test. WBS is considered an objective measurement of meat tenderness.

To address the cost-effectiveness of sensory panels, researchers have sought to establish correlations between Warner-Bratzler shear force (WBS) values and consumer-perceived

tenderness. A study examined the ability of consumers to discern different levels of beef tenderness determined by WBS measurements. *Longissimus thoracis* samples were prepared from the 8th to 13th thoracic vertebrae, vacuum-packaged, aged at 2°C for either 4 or 8 days, and then frozen at -25°C until testing. Samples were thawed at 2°C for 24 hours before being roasted in an electric convection oven preheated to 210°C until reaching an internal temperature of 70°C, monitored using a thermocouple.

For sensory evaluation, steaks were trimmed, and 1.3 × 1.3 × 1.9 cm cubes were presented hot to panelists. For shear force determination, steaks were stored overnight at 2°C post-cooking before at least six 1.27 cm diameter cores were extracted parallel to the muscle fibers. The cores were sheared perpendicular to the fibers using an Instron device equipped with a Warner–Bratzler shear head at a crosshead speed of 200 mm/min. The maximum shear force (in N) was recorded for analysis.

A point scale ranging from 1 (very tough) to 5 (very tender) was used. The study involved 220 participants who evaluated 60 samples of *longissimus thoracis*. The WBS range (23.0-72.6 N) was divided into five tenderness categories. The authors discovered that consumers had difficulty discriminating between category 1 (WBS > 62.6 N) and category 2 (WBS: 52.8-62.6 N). However, consumers showed a stronger ability to distinguish category 5 (WBS < 33.0 N). As a result, the authors combined category 1 and 2 to form a single category and combined category 4 with 5. With the establishment of these three new groups, 55.6% of consumers were able to differentiate between tough and intermediate/tender meat, while 62% were able to distinguish between tender and intermediate/tough meat. Based on their findings, the authors concluded that WBS values above 52.7 N and below 42.9 N could reliably classify beef as tender or tough (Destefanis et al., 2008).

For lamb, tenderness benchmarks are notably lower than beef, reflecting species-specific differences in muscle structure and fat distribution. Studies indicate that lamb meat with WBS values below approximately 44 N is perceived as "very tender" by consumers, with American lamb loin chops averaging around 20.1 N, well below this threshold (Carvalho Neto, 2011). These findings demonstrate that lamb is naturally tender compared to beef, reinforcing the suitability of using WBS thresholds to classify lamb tenderness and support consumer satisfaction.

2.6.3 Water-holding Capacity

Water is a major component of lean muscle, constituting approximately 75% of its composition, and it is distributed within the muscle structure and cells (Huff-Lonergan and Lonergan. 2005). The amount and distribution of water can be influenced by postmortem metabolism and handling. Extracellular water is more readily lost compared to intracellular water. Water molecules have a dipolar characteristic, which promotes their binding to proteins. Bound water refers to water that is tightly associated with proteins, while free water is unconstrained and can move freely without chemical interaction with meat proteins. Immobilized water refers to water that weakly interacts with proteins and has a lower chemical affinity. During the conversion of muscle to meat, the presence of more immobilized water leads to a higher water-holding capacity of the meat, which is desirable.

As the pH of the muscle decreases and reaches the isoelectric point of proteins, proteins have a stronger attraction to each other than to water, resulting in a decrease in the amount of water that proteins can hold. This leads to a reduction in the space within the myofibril, as well as protein denaturation of myosin heads, causing the thick and thin filaments to draw closer together. Consequently, the muscle's capacity to hold water is limited. These changes result in the

release of expressible moisture and the formation of a pale color due to the exposure of sarcoplasmic proteins (Scheffler and Gerrard. 2007; Offer et al., 1989).

2.7 References

Aberle, E. D., Forrest, J. C., Gerrard, D. E., & Mills, E. W. (2012). *Principles of Meat Science* (5th ed). Kendall Hunt Publishing Company, Dubuque, IA.

Adeyemi, K. D., & Sazili, A. Q. (2014). Efficacy of carcass electrical stimulation in Meat Quality Enhancement: A Review. *Asian-Australasian Journal of Animal Sciences*, *27*(3), 447–456. https://doi.org/10.5713/ajas.2013.13463

AMPC. (n.d.-a). Meat Technology update. https://meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_97-4.pdf

American Meat Science Association (2016). Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat., 2(1.02), 1–105. https://meatscience.org/docs/default-source/publications-resources/research-guide/amsa-research-guidelines-for-cookery-and-evaluation-1-02.pdf?sfvrsn=4c6b8eb3_2

Bendall, J. R., Ketteridge, C. C., & George, A. R. (1976). The electrical stimulation of beef carcasses. *Journal of the Science of Food and Agriculture*, *27*(12), 1123–1131. https://doi.org/10.1002/jsfa.2740271207

Bowker, B. C., Wynveen, E. J., Grant, A. L., & Gerrard, D. E. (1999). Effects of electrical stimulation on early postmortem muscle ph and temperature declines in pigs from different genetic lines and halothane genotypes. *Meat Science*, *53*(2), 125–133. https://doi.org/10.1016/s0309-1740(99)00043-1

Carlin, K. R., Huff-Lonergan, E., Rowe, L. J., & Lonergan, S. M. (2006). Effect of oxidation, pH, and ionic strength on calpastatin inhibition of μ- and M-calpain. *Journal of Animal Science*, *84*(4), 925–937. https://doi.org/10.2527/2006.844925x

Carvalho Neto, F. A. de. (2011). *Instrument evaluation of lamb carcass yield and quality characteristics* (Master's thesis). Colorado State University ProQuest Dissertations & Theses, 2011. 1492457.

Cetin, O., Bingol, E. B., Colak, H., & Hampikyan, H. (2012). Effects of electrical stimulation on meat quality of lamb and Goat Meat. *The Scientific World Journal*, *2012*, 1–9. https://doi.org/10.1100/2012/574202

Cross, H. R., West, R. L., & Dutson, T. R. (1981). Comparison of methods for measuring sarcomere length in beef semitendinosus muscle. *Meat Science*, *5*(4), 261–266. https://doi.org/10.1016/0309-1740(81)90016-4

Destefanis, G., Brugiapaglia, A., Barge, M. T., & Dal Molin, E. (2008). Relationship between beef consumer tenderness perception and Warner–Bratzler Shear Force. *Meat Science*, *78*(3), 153–156. https://doi.org/10.1016/j.meatsci.2007.05.031

Devine, C. (2009). Advantages of electrical stimulation. *Meat Science*, *83*(3), 584–585. https://doi.org/10.1016/j.meatsci.2009.06.012

Dikeman, M. E., Hunt, M. C., Addis, P. B., Schoenbeck, H. J., Pullen, M., Katsanidis, E., & Yancey, E. J. (2003). Effects of postexsanguination vascular infusion of cattle with a solution of saccharides, sodium chloride, and phosphates or with calcium chloride on quality and sensory traits of steaks and ground beef. *Journal of Animal Science*, *81*(1), 156–166. https://doi.org/10.2527/2003.811156x

Dransfeld, E. (1994). Modeling post-mortem tenderization - V: Inactivation of calpains. *Meat Science*, *37*(3), 391–409. https://doi.org/10.1016/0309-1740(94)90055-8

Ertbjerg, P., & Puolanne, E. (2017). Muscle structure, sarcomere length and influences on Meat Quality: A Review. *Meat Science*, *132*, 139–152. https://doi.org/10.1016/j.meatsci.2017.04.261

Farm Animal Welfare Council (FAWC), Report on the Welfare of Farmed Animals at Slaughter or Killing Part 1: Red Meat Animals (2003). Defra Publications. Retrieved August 14, 2023, from https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/325241/FAWC_report_on_the_welfare_of_farmed_animals_at_slaughter_or_killing_part_one_red_meat_animals.pdf.

Farouk, M. M., & Price, J. F. (1994). The effect of post-exsanguination infusion on the composition, exudation, color and post-mortem metabolic changes in lamb. *Meat Science*, *38*(3), 477–496. https://doi.org/10.1016/0309-1740(94)90072-8

Farouk, M. M., Price, J. F., Salih, A. M., & Burnett, R. J. (1992). The effect of postexsanguination infusion of beef on composition, tenderness, and functional properties. *Journal of Animal Science*, *70*(9), 2773–2778. https://doi.org/10.2527/1992.7092773x

Guo, W., & Greaser, M. L. (2017). Muscle structure, proteins, and Meat Quality. New Aspects of Meat Quality, 13–31. https://doi.org/10.1016/b978-0-08-100593-4.00002-3

Hopkins, D. L., & Thompson, J. M. (2001). Inhibition of protease activity 2. degradation of myofibrillar proteins, myofibril examination and determination of free calcium levels. *Meat Science*, *59*(2), 199–209. https://doi.org/10.1016/s0309-1740(01)00071-7

Huff Lonergan, E., Zhang, W., & Lonergan, S. M. (2010). Biochemistry of postmortem muscle — lessons on mechanisms of meat tenderization. *Meat Science*, *86*(1), 184–195. https://doi.org/10.1016/j.meatsci.2010.05.004

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science*, *71*(1), 194–204. https://doi.org/10.1016/j.meatsci.2005.04.022

Hwang, I. H., & Thompson, J. M. (2001). The effect of time and type of electrical stimulation on the calpain system and meat tenderness in beef longissimus dorsi muscle. *Meat Science*, *58*(2), 135–144. https://doi.org/10.1016/s0309-1740(00)00141-8

Hwang, K., Claus, J. R., Jeong, J. Y., Hwang, Y.-H., & Joo, S.-T. (2022). Vascular rinsing and chilling carcasses improves meat quality and food safety: A Review. *Journal of Animal Science and Technology*, *64*(3), 397–408. https://doi.org/10.5187/jast.2022.e29

James, S. J., & James, C. (2002). Effect of refrigeration on texture of meat. In *Meat Refrigeration* (pp. 43–69). essay, Woodhead.

Kethavath, S. C., Hwang, K., Mickelson, M. A., Campbell, R. E., Richards, M. P., & Claus, J. R. (2021). Vascular infusion with concurrent vascular rinsing on color, tenderness, and lipid oxidation of Hog Meat. *Meat Science*, 174, 108409. https://doi.org/10.1016/j.meatsci.2020.108409

Kethavath, S. C., Moreira, L. da, Hwang, K., Mickelson, M. A., Campbell, R. E., Chen, L., & Claus, J. R. (2022). Vascular rinsing and Chilling Effects on meat quality attributes from cull dairy cows associated with the two lowest-valued marketing classes. *Meat Science*, *184*, 108660. https://doi.org/10.1016/j.meatsci.2021.108660

Koohmaraie, M, Shackelford, S. D. (1991). Effect of calcium chloride infusion on the tenderness of lambs fed a beta-adrenergic agonist. *Journal of Animal Science*, *69*(6), 2463. https://doi.org/10.2527/1991.6962463x

Koohmaraie, M. (1992). The role of ca2+-dependent proteases (calpains) in post mortem proteolysis and Meat Tenderness. *Biochimie*, *74*(3), 239–245. https://doi.org/10.1016/0300-9084(92)90122-u

Koohmaraie, M., Crouse, J. D., & Mersmann, H. J. (1989). Acceleration of postmortem tenderization in ovine carcasses through infusion of calcium chloride: Effect of concentration and Ionic strength. *Journal of Animal Science*, *67*(4), 934–942. https://doi.org/10.2527/jas1989.674934x

Lansdell, J. L., Miller, M. F., Wheeler, T. L., Koohmaraie, M., & Ramsey, C. B. (1995). Postmortem injection of calcium chloride effects on beef quality traits. *Journal of Animal Science*, *73*(6), 1735–1740. https://doi.org/10.2527/1995.7361735x

Maddock, K. R. (2005). *Effects of oxidation, ph, and ionic strength on inhibition of mu- and M-calpain by Calpastatin* (dissertation). Iowa State University Digital Repository.

Mancini, R. A., & Hunt, M. C. (2005). Current research in Meat Color. *Meat Science*, *71*(1), 100–121. https://doi.org/10.1016/j.meatsci.2005.03.003

Marsh, B. B., & Leet, N. G. (1966). Studies in meat tenderness. III. the effects of cold shortening on tenderness. *Journal of Food Science*, *31*(3), 450–459. https://doi.org/10.1111/j.1365-2621.1966.tb00520.x

Mickelson, M. A. (2018). *Carcass chilling method effects on instrumental color and tenderness of bison, lamb, and sow meat* (Master's thesis). University of Wisconsin-Madison

Mickelson, M. A., & Claus, J. R. (2020). Carcass chilling method effects on color and tenderness of bison meat. *Meat Science*, *161*, 108002. https://doi.org/10.1016/j.meatsci.2019.108002

Mickelson, M. A., Warner, R. D., Polkinghorne, R. J., Seman, D. L., Crump, P. M., & Claus, J. R. (2025). Carcass chilling method and electrical stimulation effects on meat quality and color in lamb. *Meat Science*, *219*, 109662. https://doi.org/10.1016/j.meatsci.2024.109662

Miller, M. (2007). Dark, Firm and Dry Beef. https://fyi.extension.wisc.edu/wbic/files/2011/04/Dark-Firm-and-Dry-Beef.pdf

Nguyen, T., Kim, S., & Kim, J. G. (2019). Diffuse reflectance spectroscopy to quantify the met-myoglobin proportion and meat oxygenation inside of pork and beef. *Food Chemistry*, *275*, 369–376. https://doi.org/10.1016/j.foodchem.2018.09.121

Novaković, S., & Tomašević, I. (2017). A comparison between Warner-Bratzler shear force measurement and texture profile analysis of meat and Meat Products: A Review. *IOP Conference Series: Earth and Environmental Science*, *85*, 012063. https://doi.org/10.1088/1755-1315/85/1/012063

Offer, G., Knight, P., Jeacocke, R., Almond, R., Cousins, T., & Elsey, J. (1989). The Structural Basis of the Water-Holding, Appearance and Toughness of Meat and Meat Products. *Food Structure*, *8*(1), 151–170.

Page, J. K., Wulf, D. M., & Schwotzer, T. R. (2001). A survey of beef muscle color and pH. *Journal of Animal Science*, *79*(3), 678. https://doi.org/10.2527/2001.793678x

Polidori, P., Trabalza Marinucci, M., Fantuz, F., Renieri, C., & Polidori, F. (2000). Tenderization of Wether lambs meat through pre-rigor infusion of calcium ions. *Meat Science*, *55*(2), 197–200. https://doi.org/10.1016/s0309-1740(99)00143-6

Rees, M. P., Trout, G. R., & Warner, R. D. (2002). Effect of calcium infusion on tenderness and ageing rate of pork M. Longissimus thoracis et lumborum after accelerated boning. *Meat Science*, *61*(2), 169–179. https://doi.org/10.1016/s0309-1740(01)00181-4

Scheffler, T. L., & Gerrard, D. E. (2007). Mechanisms controlling pork quality development: The biochemistry controlling postmortem energy metabolism. *Meat Science*, *77*(1), 7–16. https://doi.org/10.1016/j.meatsci.2007.04.024

USDA. (2019). *Fooddata Central Search Results: Lamb, New Zealand, imported, loin, boneless, separable lean and fat, raw*. FoodData Central. https://fdc.nal.usda.gov/fdc-app.html#/food-details/172635/nutrients

Veiseth, E., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2001). Effect of postmortem storage on Mu-calpain and M-calpain in ovine skeletal muscle. *Journal of Animal Science*, *79*(6), 1502. https://doi.org/10.2527/2001.7961502x

Warner, R. D., Wheeler, T. L., Ha, M., Li, X., Bekhit, A. E.-D., Morton, J., Vaskoska, R., Dunshea, F. R., Liu, R., Purslow, P., & Zhang, W. (2022). Meat tenderness: Advances in biology, biochemistry, molecular mechanisms and new technologies. *Meat Science*, *185*, 108657. https://doi.org/10.1016/j.meatsci.2021.108657

Webb, E. C. (2014). Goat meat production, composition, and quality. *Animal Frontiers*, *4*(4), 33–37. https://doi.org/10.2527/af.2014-0031

Wheeler, T. L., Koohmaraie, M., Lansdell, J. L., Siragusa, G. R., & Miller, M. F. (1993). Effects of postmortem injection time, injection level, and concentration of calcium chloride on beef quality traits. *Journal of Animal Science*, *71*(11), 2965–2974. https://doi.org/10.2527/1993.71112965x

Chapter 3

Influence of Carcass Vascular Rinsing with Calcium Chloride on Non-Electrically Stimulated and Electrically Stimulated Carcasses on Meat Quality of Lambs

Running head: Vascular Rinsing with Calcium Chloride on Meat Quality

3.1. Abstract

The study was to determine the effects of vascular rinsing carcasses with a calcium chloride solution on lamb meat quality. Lambs (n=40) of various breeds, age (6-9 months) were assigned to vascular rinse treatments (TRT) that included:(RC= Rinse & Chill® solution; saccharides, phosphates), (CA= 0.3M $CaCl_2$ + RC), (ES-CA= electrical stimulation, 800mA, 350V, followed by CA). Animals were mechanically stunned and exsanguinated prior to rinsing 10% of their live weight. Carcasses were skinned, eviscerated, and chilled (3°C, 24 h). Carcass temperature and pH were recorded (semimembranosus, SM) from 1h to 20h postmortem (PM). At 24 h PM, the longissimus dorsi (LD), SM, and triceps brachii (TB) were excised, vacuum packaged or overwrapped in oxygen-permeable film. Calcium and phosphorus content in the muscles were determined. Color measurements (CIE L^*, CIE a^*, chemical states of myoglobin) were determined during storage (3 and 7 d) PM. Purge, Warner-Bratzler shear force (WBS), and cook loss; cooked chops 68.3°C internal (3 and 7 d) PM; LD, SM, and TB were determined. Data was analyzed as a split-split plot design with means ($P<0.05$) separated using PROC MIXED (SAS Institute).

Live animal and carcass weights were not different ($P>0.05$) among the treatments. For carcass pH, ES-CA was lower ($P<0.05$) than CA and RC, while CA was lower ($P<0.05$) than RC.

In the LD, calcium had a lower (P<0.05) concentration in RC than CA and ES-CA. In the TB, there was a lower (P<0.05) concentration of calcium in RC than in ES-CA. In the SM, calcium had a lower (P<0.05) concentration in RC than CA and ES-CA. No treatment differences were found in phosphorous concentrations. RC had greater CIE L^*, CIE a^*, Chroma C, and oxymyoglobin, and lower values for metmyoglobin (P<0.05). In the LD and SM muscles, RC had less (P<0.05) purge than CA and ES-CA. In the TB muscle, purge was greater (P<0.05) in ES-Ca than RC and CA. RC had lower (P<0.05) cook loss than ES-CA. The LD in CA and ES-CA had lower (P<0.05) WBS than RC. With ES-CA, the SM exhibited the lowest (P<0.05) WBS. RC had the greatest WBS at day 3 (P<0.05), with no differences (P>0.05) found between TRT on day 7.

The implications of vascularly delivering calcium chloride throughout the carcass on meat quality are noteworthy, particularly on shear force reduction, suggesting the potential to enhance tenderness. More rapid chilling to reduce the effect of the rapid drop in pH may benefit meat color and moisture retention.

Key words: calcium chloride, carcass vascular rinsing, electrical Stimulation, lamb, meat quality

3.2 Introduction

Introducing calcium chloride into carcasses before rigor mortis onset holds promise for enhancing meat quality and tenderness. This approach aims to activate crucial enzymes, μ-calpain and m-calpain, which play key roles in breaking down myofibrillar and cytoskeletal proteins postmortem (Huff Lonergan et al., 2010). Activation of calpains depends on the presence of calcium, yet sometimes, intramuscular calcium levels may be insufficient for full activation. The longstanding practice of using calcium chloride as a meat tenderizer stems from

its ability to elevate intramuscular calcium ions, thereby accelerating the enzymatic breakdown of essential myofibrillar proteins. This process disrupts muscle fiber structure, ultimately enhancing tenderness. Prior studies have demonstrated the effectiveness of infusing lambs with a 0.3 M solution of CaCl2 (10% live weight) via intra-arterial infusion, resulting in reduced shear force at various postmortem intervals 1, 7, 14 days; (Koohmaraie et al., 1991).

It is worth noting that not all studies have consistently shown an improvement in meat tenderness with calcium chloride infusion. For instance, in cattle vascularly infused with 0.3M $CaCl_2$ at 10% live weight using Rinse & Chill® (MPSC Inc), Warner-Bratzler shear force values for the *Longissimus lumborum* were found to be higher than other treatments (Dikeman et al., 2003). It was proposed that incorporating carcass electrical stimulation prior to calcium chloride infusion could deplete readily available ATP and exhaust the muscular system, thereby preventing extensive contraction and toughening (Koohmaraie et al., 1989). Carcass electrical stimulation entails applying electrical impulses to muscles and is renowned for expediting postmortem pH decline by triggering skeletal muscle contractions. Research has consistently demonstrated its positive impact on meat quality across various meat types, including turkey, lamb, beef, and veal (Aberle et al., 2012).

The emergence of Rinse & Chill® technology (MPSC Inc., Hudson, United States) has furthered the commercial viability of calcium chloride infusion into carcasses, providing avenues for continued exploration and application of such methods. Rinse & Chill® technology, widely adopted in lamb and beef packing plants, involves circulating a chilled solution through the vasculature during exsanguination to expel warm residual blood, thus accelerating carcass chilling and improving yields. Comprising mostly water (98.5%), with additional dextrose,

maltose, and phosphates, the isotonic solution has been studied for its effects on various red meat species, including beef, lamb, bison, and recently, pork.

Previous studies on Rinse & Chill® technology in lamb and bison have shown improvements in tenderness and red meat color (Mickelson & Claus. 2020; Fowler et al., 2017). Kethavath et al. (2021) explored its effects on electrically stunned market hogs, suggesting quality enhancements even with rapid pH decline, particularly in suboptimal harvest facility temperature conditions. This study aims to expand upon previous findings by examining how electrical stimulation and the incorporation of calcium chloride into the rinse solution affect lamb carcasses.

It was hypothesized that administering electrical stimulation prior to calcium chloride infusion could mitigate extensive toughening, increase rinse solution distribution, and the presence of calcium would lead to decreased shear force and enhanced meat tenderness.

3.3 Materials and Methods

Market Lambs (n=40, average live weight 59.42 ±29.48 kg, age 6-9 months, commercial crossbred, Dorset, Hampshire, Suffolk, Polypay, Southdown) were harvested across three weeks in October and November 2023 under inspection by the United States Department of Agriculture (USDA) in the Meat Science and Muscle Biologics Discovery Building at UW – Madison. Lambs were procured from the Arlington Agricultural Research Station Sheep Research Unit and private commercial herds in Wisconsin. Free water access and limited feed (12 h before harvest) were provided before harvest.

Vascular rinse treatments

This study employed three treatments, each involved specific vascular rinsing applications, with one utilizing electrical stimulation of the carcass before rinsing. The vascular rinsing process entailed inserting a catheter into the heart and rinsing the carcass at 10% of its live weight.

RC (Rinse & Chill®) Treatment. Vascular rinsing was performed immediately after exsanguination using a proprietary Rinse & Chill® solution. The solution was prechilled (3.0°C) and consisted of 98.5% water with the balance being dextrose, phosphates, and maltose.

CA ($CaCl_2$ + RC) Treatment. Vascular rinsing included the addition of 0.3M $CaCl_2$ to the proprietary Rinse & Chill® solution. The carcass was vascularly rinsed immediately after exsanguination.

ES-CA (Electrical Stimulation + CA) Treatment. Carcass electrical stimulation was administered immediately after exsanguination. Electrical stimulation was applied for two 30-second consecutive applications (800mA current, peak 350V, 0.5ms pulse width, and a 68ms pulse period (15Hz) (StimTech Pty Ltd, Coopers Plains Australia, Model Multifunctional Stimulator / Immobiliser). The electrical stimulation unit utilized clamp and probe-style electrodes in which one lead was clamped to the carcass nose while the other lead was inserted into the rectum. After carcass stimulation, vascular rinsing was conducted with the addition of 0.3M $CaCl_2$ to the Rinse & Chill® solution.

Carcass storage, pH, and temperature

Carcasses were then air-chilled at 3.0°C overnight. Carcass temperature and pH were continuously recorded (*Semimembranosus*) from 1 to 20 h postmortem with samples being taken

every 30 minutes. (MadgeTech, Inc, Warner, NH, Model pHTemp2000, 900988-00; pH Sensor InLab® Solids Go-ISM Material #:51343156). The pH electrodes were calibrated using standard buffer solutions (pH 4 and 7) and calibrating the meter to those endpoints before use.

Carcass fabrication and sample preparation

At 24 h postmortem, the *Longissimus thoracis et lumborum* (LD), *Semimembranosus* (SM), and *Triceps brachii* (TB) were excised from the carcass. The *Longissimus thoracis et lumborum* was removed from the carcass between the 8th thoracic vertebra and the last lumbar vertebra. Muscles were excised and cut to a thickness of 1.27 cm with approximately 0.1 cm of subcutaneous fat remaining. Samples were then vacuum packaged (VAC), or overwrapped. Chops (2 per treatment) for display were overwrapped in oxygen-permeable polyvinyl chloride film (PVC; oxygen transmission rate, OTR: 22,480 cm^3/m^2/24 h at 23 °C; water transmission rate = 496 g/m2/24 h at 37.8 °C and 90% relative humidity; product code 75003815, AEP Industries Inc., South Hackensack, NJ) using a single-roll over-wrapper on a plastic tray (product code 38210030, Bunzl-Koch Supplies, Kansas City, MO). The remaining muscle samples were individually VAC packaged (vacuum setting, 10/10; model 2100-C; UltraVac-dual chamber; Koch Equipment LLC., Kansas City, MO) in plastic pouches (2.7 mil thick, OTR 3-6cc/ m^2 /24h atm @ 4 °C, 0 % relative humidity, blend of very low-density polyethylene and ethylene vinyl acetate copolymer, 18 x 30 cm, product code 9KN81, Sealed Air Corporation). VAC samples for Purge, Cook Loss and Warner-Bratzler shear force were stored in the dark (1-2 °C) and analyzed at days 3 and 7 postmortem. VAC samples for calcium and phosphorus content analysis were stored frozen (-17.78°C).

Calcium, phosphorus, moisture, and fat

Concentrations of calcium and phosphorus in the LD, TB, and SM muscles were analyzed (Deibel Laboratories, Manhattan, United States) using inductively coupled plasma - optical emission spectrometry (AOAC 985.01 modified). Samples were ground (Robot Coupe®, USA. Inc. Model Blixer® 2), and VAC before shipping.

Color display

PVC-wrapped muscles were continuously displayed in an open-topped refrigerated (1-2 °C) display case (model THG7SG; Fricon, Portugal) under fluorescent lighting (40 watt, F40/CWX, Sylvania Cool White Deluxe, Danvers, MA) that provided approximately 1076 lux. A temperature logger (model: QuadTemp 2000; ThermoWorks; American Fork, UT) was placed in each display case to record every 5 min continuously for 7 d.

Color measurement and myoglobin determination

Color measurements (CIE $L^*a^*b^*$; reflectance estimators of the chemical states of myoglobin) were obtained on days 3 and 7 PM. Six colorimeter measurements were obtained on each LD, SM and TB sample at every time interval using a handheld spectrophotometer (CM-600d; D65 light source; Konica Minolta Inc, Japan). The spectrophotometer was calibrated each day using the white calibration cap (CM-A177), overwrapped with PVC. The chemical states of myoglobin were estimated using AMSA's Meat Color Measurement Guidelines: metmyoglobin (MMb, Equation 2), deoxymyoglobin (DMb, Equation 3), and oxymyoglobin (OMb, Equation 4) (AMSA, 2012). Chroma C was estimated using the formula: $\sqrt{(a*)^2 + (b*)^2}$. The hue angle was estimated using the formula: $\tan^{-1}(\frac{b*}{a*})$.

Purge

Purge determination was conducted on fresh, non-frozen samples on both days 3 and 7 postmortem (PM). For purge determination, each muscle was initially weighed within its vacuum packaging (VAC) bag. The average weight of an unused bag was used to determine the net weight. The subsequent steps involved removing the muscle from the VAC bag, patting it with paper towels to eliminate excess visible moisture on the external surface, and then reweighing it. The percentage of purge amount was determined by the difference between the initial weight of the muscle along with the VAC bag and the weight of the muscle after being patted dry.

Cook loss and Warner-Bratzler shear force

To determine cook loss (CL) and Warner-Bratzler shear force (WBS), individual muscles were first weighed and then arranged on cooking racks. Each rack accommodated either 9 loin chops with a thickness of 1 inch (LD), 3 SM muscles, or 3 TB muscles, with each treatment represented. Chops were cycled through different positions on the cooking racks to ensure uniform distribution in the oven. The cooking process was carried out in an ALTO-SHAAM COMBITHERM Oven (Model: CTX4-10E) at a constant temperature of 350°C using the convection mode setting. During the cooking process, all samples had a thermocouple (model QuadTemp 2000; ThermoWorks; American Fork, UT) inserted in the geometric center to monitor the internal temperature. The samples were removed from the oven once they reached an internal temperature of 68.33°C. After cooking, the samples were placed on metal cooling racks at room temperature for 1 hour. Subsequently, each chop was individually reweighed for determination of cook loss using the formula ((raw weight – cooked weight)/raw weight × 100).

For the Warner-Bratzler shear (WBS) analysis, six strips were obtained from each chop, measuring 1 cm in thickness, 1 cm in width, and at least 2.5 cm in length. Strips were cut parallel to the muscle fibers using a two-bladed scalpel with the blades spaced (1.0 cm apart). The strips were subjected to shearing using a G-R Shear Machine (Model: GR-151; G-R Electric Manufacturing Company LLC; Manhattan, KS). The shearing was performed once through a v-notch blade, positioned perpendicularly to the muscle fibers at the center of each strip. The average of the six shear results from the samples were calculated and used for subsequent statistical analysis.

3.4 Results and Discussion

Live animal weights were not different ($P > 0.05$) among the treatments (LSMeans: 31.2 kg, CA; 31.8 kg, ES-CA; 31.0 kg, RC; S.E. 1.29). Similarly, hot carcass weights were not different ($P > 0.05$) among the treatments (LSMeans: 31.2 kg, CA; 31.8 kg, ES-CA; 31.0 kg, RC; S.E. 1.29). Chilled carcass weights followed the same trend, with no differences ($P > 0.05$) observed between treatments. However, carcass shrink, defined as the percentage difference between hot carcass weight and chilled carcass weight, also did not differ ($P > 0.05$) among treatments (LSMeans: 2.17%, CA; 2.30%, ES-CA; 2.07%, RC; S.E. 0.178). These findings indicate that the treatments did not influence live animal weights, hot carcass weights, or carcass shrink, demonstrating uniformity in carcass performance across the three groups.

pH Decline

For carcass pH, ES-CA was lower ($P<0.05$) than CA and RC, while CA was lower ($P<0.05$) than RC (Figure 1). When the pH was first determined, ES-CA was already lower than the other treatments likely from the effects of electrical stimulation applied immediately after

exsanguination. Electrical stimulation of the carcass caused the muscles to repeatedly contract and relax which hastened postmortem glycolysis, leading to a pH decline through the rapid utilization of muscle glycogen (Pearson & Dutson. 1985).

Previous research on lamb has demonstrated that electrical stimulation treatments led to a faster pH decline compared to non-stimulated treatments. The early application of electrical stimulation, prior to evisceration, caused carcass pH to decrease more rapidly than in both the control and Rinse & Chill® treated carcasses (Mickelson et al., 2025). This aligns with the observation that the ES-CA treatment had a lower pH compared to RC and CA treatments. This effect is attributed to accelerated postmortem glycolysis, driven by muscle contractions from electrical stimulation, leading to faster muscle glycogen depletion and lactic acid production, mirroring the explanation for the pH decline seen with ES-CA.

Additionally, CA likely exhibited a lower pH compared to RC due to calcium's capability to stimulate muscle contraction (Wicks et al., 2022). Vascularly rinsing with the solution containing calcium would elevate the sarcoplasmic level of calcium above the resting state potential. Calcium would bind to troponin which would remove tropomyosin from myosin’s binding site on actin thereby causing initiating the contraction cycle. Muscle contraction is an energy dependent reaction utilizing ATP (Aberle et al., 2012).

Previous research on lamb supports the role of vascular infusion of calcium chloride in facilitating a rapid drop in pH and stimulating postmortem glycolysis. When lamb carcasses were infused with 0.3M calcium chloride, the longissimus dorsi reached its ultimate pH faster compared to control lambs and those infused with 0.5M zinc chloride or water. Calcium-infused carcasses achieved ultimate pH within 2.5 hours postmortem. However, no differences were observed in the ultimate pH values between the treatments, indicating that the effect of calcium

infusion primarily influenced the rate of pH decline rather than the final pH level (Bekhit et al., 2005).

Also, since vascular rinsing removes more of the blood than conventional bleeding (Hwang et al., 2022), then a more rapid shift to more anaerobic metabolism would likely occur. Residual blood allows muscle to remain under aerobic conditions for a greater period. With the shift to anaerobic conditions, glycogen would be metabolized to lactic acid, thereby influencing pH levels. These results support those of Dikeman et al. (2003), who noted that infusing beef carcasses with 0.3M calcium chloride led to a more rapid pH decline compared to control carcasses.

Carcass temperature

For carcass temperature, the RC treatment was lower ($P<0.05$) than the CA and ES-CA treatments, while the CA and ES-CA treatments did not differ ($P>0.05$) (Figure 2). The CA and ES-CA treatments likely generated more metabolic heat in the early postmortem period, leading to elevated carcass temperatures compared to the RC treatment. Elevated sarcoplasmic calcium from electrical stimulation and vascular rinsing can enhance muscle contraction and overall muscle metabolism. An increased rate of metabolism generates additional heat that must be dissipated (Wicks et al., 2022).

Calcium and phosphorus content in muscle

For calcium concentration, a treatment x muscle effect was observed (Table 1). In the LD muscle, calcium had a lower ($P<0.05$) concentration in the RC treatment than CA and ES-CA. In the TB muscle, there was a lower ($P<0.05$) concentration of calcium in the RC treatment than in ES-CA. The CA treatment did not differ ($P>0.05$) between RC and ES-CA in calcium

concentration in the TB muscle. The elevated calcium levels observed in the TB of ES-CA may be attributed to the capacity of electrical stimulation to fatigue the muscles, potentially facilitating the dispersion of rinse solution within the muscle tissue. In the SM muscle, calcium had a lower ($P<0.05$) concentration in the RC treatment than CA and ES-CA. No treatment differences were found in phosphorous concentrations. The SM muscle had greater ($P<0.05$) phosphorous concentrations than the LD and TB. The LD had higher ($P<0.05$) phosphorous concentrations than the TB muscle.

In contrast to the findings of Hwang et al. (2022), who found phosphorus concentrations of 2,079 mg/kg in vascularly rinsed beef LD muscles and 2,113 mg/kg in non-rinsed LD muscles, our study observed phosphorus concentrations of 1,838.0 mg/kg in rinsed lamb carcasses. These observations align with the USDA's nutrient database of Lamb, New Zealand, imported, square-cut shoulder, separable lean only, raw which had a phosphorus concentration of 1,750 mg/kg (USDA. 2019).

Instrumental color

There were not any two-way or three-way treatment interactions ($P>0.05$) with muscle or display time (Table 2). RC had greater CIE L^*, CIE a^* and Chroma C than CA and ES-CA ($P<0.05$, with no differences ($P>0.05$) between CA and ES-CA. RC also had a greater percentage of OMb, and a lower percentage of MMb than CA and ES-CA ($P<0.05$); with no differences between CA and ES-CA. No differences in hue angle and percentage of DMb were found between treatments ($P>0.05$).

The lower CIE L^* values in both CA and ES-CA may be attributed to the muscles being in a highly contracted state, reducing the amount of reflective light. Despite the treatment

differences in CIE L^* and a^* values, fresh lamb color is generally considered acceptable by consumers when the a^* value is at least 9.5 and the L^* value is at least 34 (Khliji et al., 2010). All three rinse treatments have surpassed this threshold for both CIE L^* and CIE a^*. The greater amount of metmyoglobin in the CA and ES-CA treatments may be attributed to the rapid drop in carcass pH while temperatures were still high. Rapid pH drop can lead to meat having unfavorable texture and water-binding characteristics, akin to PSE pork. This can encourage the formation of metmyoglobin. Moreover, when there's a swift decline in pH coupled with increased muscle temperature, it creates conditions conducive to protein denaturation. In a previous study, lambs infused with calcium chloride had a higher metmyoglobin accumulation rate, and lower L^*, a^*, and b^* color measurements (Bekhit et al., 2005).

The SM muscle had lower CIE L^* ($P<0.05$) than the LD and TB, with no difference ($P>0.05$) between LD and TB. At day 3 PM, CIE a^* was lower ($P<0.05$) in the LD compared to the TB and SM, with no difference ($P>0.05$) between the TB and SM (Table 3). On day 7 PM, CIE a^* was higher ($P<0.05$) in the TB compared to the LD and SM, with no difference ($P>0.05$) between the LD and SM. At day 3 PM, hue was lower ($P<0.05$) in the SM than in the LD and TB, while there was no difference ($P>0.05$) between the LD and TB. At day 7 PM, hue was higher ($P<0.05$) in the LD compared to TB and SM, while it was higher ($P<0.05$) in the SM compared to TB. At Day 3 PM, Chroma C was higher ($P<0.05$) in the TB compared to the SM and LD, while the SM muscle was higher ($P<0.05$) than the LD. At Day 7 PM, Chroma C was higher ($P<0.05$) in the TB muscle compared to the SM and LD, with no difference ($P>0.05$) between the SM and LD. At day 3 PM, OMb was higher ($P<0.05$) in the TB compared to LD and SM, with no difference ($P>0.05$) observed between LD and TB. At day 7 PM OMb was higher ($P<0.05$) in the TB compared to LD and SM, with no difference ($P>0.05$) observed between LD

and TB. DMb was higher ($P<0.05$) in the TB muscle compared to LD and SM, with no difference ($P>0.05$) between LD and SM. At day 3 PM, the TB had lower metmyoglobin ($P<0.05$) than LD and SM, with no difference ($P>0.05$) between LD and SM. At day 7 PM, the TB had lower metmyoglobin ($P<0.05$) than LD and SM, with no difference ($P>0.05$) between LD and SM.*Purge and cook loss*

For purge, a treatment x muscle interaction was observed ($P<0.05$, Table 4). In the LD muscle, purge was greater ($P<0.05$) in ES-CA than CA and RC, and greater ($P<0.05$) in CA than RC. In the SM muscle, purge was lower ($P<0.05$) RC CA and ES-CA; with no difference ($P>0.05$) between CA and ES-CA. In the TB muscle, purge was higher ($P<0.05$) in ES-CA compared to RC and CA; with no difference ($P>0.05$) between RC and CA.

The increased purge associated with the CA and ES-CA treatments may be attributed to a rapid pH decline. As the pH of the muscle decreases and reaches the isoelectric point of proteins, proteins have a stronger attraction to each other than to water, resulting in a decrease in the amount of water that proteins can hold. This leads to a reduction in the space within the myofibril causing the thick and thin filaments to draw closer together. Consequently, the muscle's capacity to hold water is limited. (Scheffler and Gerrard. 2007; Offer et al., 1989).

The addition of calcium in the CA and ES-CA treatments likely elevated sarcoplasmic calcium concentrations beyond the muscle’s ability to regulate contraction and relaxation. Calcium concentrations in the sarcoplasm must rise to approximately 10^{-6} to 10^{-5} moles/liter to initiate contraction (Aberle et al., 2012). In these treatments, the sarcoplasmic reticulum's capacity to resequester calcium may have been overwhelmed, prolonging calcium binding to troponin C and sustaining contraction. The calcium ion pump, which is ATP-dependent, would

have worked harder under these conditions, further depleting ATP reserves and diminishing the muscle's ability to relax.

This sustained contraction is reflected in the shorter sarcomere lengths observed in ES-CA compared to CA and RC, with CA showing shorter sarcomeres than RC (Pharino et al., 2024). Similar findings were reported in lamb carcasses infused with 0.15M calcium chloride, where sarcomeres shortened by 12% more than in controls (Farouk et al., 1992). The prolonged contraction compressed the myofibrillar structure, forcing free water out through the steric effect, while the increased ATP demand accelerated anaerobic metabolism. This led to lactic acid buildup, increased hydrogen ion concentration, and a rapid decline in pH. Together, calcium infusion and electrical stimulation amplified these effects, influencing rigor mortis onset, water-holding capacity, and muscle texture.

Cook loss was lower ($P<0.05$) in the RC treatment compared to ES-CA, with no difference ($P>0.05$) between RC and CA, as well as between CA and ES-CA. The LD muscle had higher ($P<0.05$) cook loss than the SM and TB, with no difference ($P>0.05$) between the SM and TB (Table 4). High temperatures in combination with low pH levels result in the denaturation of proteins, leading to a loss of their functionality, which in turn affects meat quality. This interaction is responsible for reduced water-holding capacity, decreased tenderization, pale color, premature browning during retail display, and sarcomere shortening. Specifically, myosin denaturation causes the myosin head to shorten and reduces filament spacing, causing water to be expelled from the muscle cells into the extracellular space (Kim et al., 2014).

Warner-Bratzler shear force (WBS)

For WBS, a treatment × day interaction was observed ($P<0.05$, Table 5). At day 3 PM, WBS was higher ($P<0.05$) in the RC treatment compared to CA and ES-CA, with no difference ($P>0.05$) observed between CA and ES-CA. At day 7 PM, there was no difference ($P>0.05$) between the three treatments. A treatment × muscle interaction was observed ($P<0.05$). In the LD muscle, WBS was higher ($P<0.05$) in the RC treatment compared to CA and ES-CA, with no difference ($P>0.05$) observed between CA and ES-CA. In the SM muscle, WBS was lower ($P<0.05$) in the ES-CA treatment compared to RC and CA, with no difference ($P>0.05$) observed between RC and CA. In the TB muscle, WBS was not different ($P>0.05$) between the three treatments.

The observed differences in WBS values should be contextualized with tenderness thresholds to assess their practical significance. Research has established that beef with WBS values below 42.87 N is perceived as "tender," and values below 32.96 N (3.36 kg) are considered "very tender" (Destefanis et al., 2008). For lamb, similar thresholds have been identified, with WBS values below approximately 44 N classified as "very tender" (Carvalho Neto, 2011). In this study, all treatments resulted in WBS values well below these thresholds, indicating that meat from all treatments would be classified as "very tender" and meet or exceed consumer expectations for tenderness.

The reduction in WBS in CA and ES-CA treatments for the LD muscle compared to RC can be attributed to increased proteolytic activity facilitated by calcium in the rinse solution. Calcium activates calpains, enzymes that degrade structural proteins such as titin and desmin, which weakens the myofibrillar matrix and improves tenderness. These results are consistent with Koohmaraie et al. (1989), who found that post-exsanguination infusion with calcium-

enriched solutions reduced shear force in ovine carcasses by accelerating postmortem proteolysis.

The SM muscle exhibited improved WBS values in the ES-CA treatment compared to RC and CA. This improvement may be attributed to the combined effects of electrical stimulation and calcium infusion. Electrical stimulation depletes ATP reserves and accelerates rigor mortis, which may facilitate better penetration of the rinse solution into muscle fibers. Similar effects were observed in bison carcasses, where Rinse & Chill® technology combined with electrical stimulation enhanced tenderness in the longissimus and triceps brachii muscles (Mickelson & Claus. 2020). The higher calcium concentrations observed in ES-CA treatments likely enhanced calpain activity in the SM muscle, leading to further reductions in shear force.

In contrast, no treatment effects were observed in the TB muscle, likely due to its high connective tissue content. Tenderness in collagen-rich muscles like TB is primarily influenced by collagen cross-links, which are not degraded by calpains. These findings are consistent with Koohmaraie et al. (1989), who reported limited impact of calcium-based treatments on collagen-dense muscles. Previous research also noted that tenderness improvements in ovine carcasses were more pronounced in muscles with lower connective tissue content (Farouk et al., 1992).

The lack of differences in WBS between treatments by day 7 PM indicates that proteolysis in the RC treatment eventually reached similar levels to those in CA and ES-CA treatments. This delayed tenderization aligns with previous research which noted that a Rinse & Chill® with the addition calcium chloride accelerated early tenderization, but extended aging allowed treatments without calcium chloride to achieve comparable tenderness (Dikeman et al., 2003). Previous studies also highlighted that the timing of proteolytic enzyme activation plays a critical role in the tenderization process (Farouk et al., 1992).

3.5 Conclusion

The results of this study illuminate the substantial implications of vascularly delivering calcium chloride throughout the carcass for enhancing meat quality. The observed reductions in shear force, particularly in the longissimus dorsi muscle, suggest a promising avenue for improving tenderness through calcium infusion. Moreover, the stimulation of postmortem muscle metabolism by calcium underscores its potential to positively influence meat characteristics. Both the CA and ES-CA treatments demonstrated further shear force reduction compared to the RC treatment, recognizing however that even the RC lamb would be considered very tender. Additionally, with the facilitation of more rapid chilling to mitigate the adverse effects of pH decline, such strategies hold promises for enhancing meat color and moisture retention when calcium is vascularly rinsed through the carcass.

3.6 References

Aberle, E. D., Forrest, J. C., Gerrard, D. E., & Mills, E. W. (2012). *Principles of Meat Science* (5th ed.). Kendall Hunt Publishing Company. Dubuque IA.

American Meat Science Association. (2012, December). AMSA Meat Color Measurement Guidelines. https://meatscience.org/docs/default-source/publications-resources/hot-topics/2012_12_meat_clr_guide.pdf

Bekhit, A. E., Ilian, M. A., Morton, J. D., Vanhanan, L., Sedcole, J. R., & Bickerstaffe, R. (2005). Effect of calcium chloride, zinc chloride, and water infusion on metmyoglobin reducing activity and fresh lamb color. *Journal of Animal Science*, 83(9), 2189–2204. https://doi.org/10.2527/2005.8392189x

Carvalho Neto, F. A. de. (2011). *Instrument evaluation of lamb carcass yield and quality characteristics* (Master's thesis). Colorado State University ProQuest Dissertations & Theses, 2011. 1492457.

Destefanis, G., Brugiapaglia, A., Barge, M. T., & Dal Molin, E. (2008). Relationship between beef consumer tenderness perception and Warner–Bratzler Shear Force. *Meat Science*, *78*(3), 153–156. https://doi.org/10.1016/j.meatsci.2007.05.031

Dikeman, M. E., Hunt, M. C., Addis, P. B., Schoenbeck, H. J., Pullen, M., Katsanidis, E., & Yancey, E. J. (2003). Effects of postexsanguination vascular infusion of cattle with a solution of saccharides, sodium chloride, and phosphates or with calcium chloride on quality and sensory traits of steaks and ground beef. *Journal of Animal Science*, 81(1), 156–166. https://doi.org/10.2527/2003.811156x

Farouk, M. M., Price, J. F., & Salih, A. M. (1992). Post-exsanguination infusion of ovine carcasses: Effect on tenderness indicators and muscle microstructure. *Journal of Food Science*, *57*(6), 1311–1315. https://doi.org/10.1111/j.1365-2621.1992.tb06844.x

Fowler, S. M., Claus, J. M., & Hopkins, D. L. (2017). The effect of applying a rinse and chill procedure to lamb carcases immediately post-death on meat quality? *Meat Science*, *134*, 124–127. https://doi.org/10.1016/j.meatsci.2017.07.015

Huff Lonergan, E., Zhang, W., & Lonergan, S. M. (2010). Biochemistry of postmortem muscle - lessons on mechanisms of meat tenderization. *Meat Science*, *86*(1), 184–195. https://doi.org/10.1016/j.meatsci.2010.05.004

Hwang, K., Claus, J. R., Jeong, J. Y., Hwang, Y.-H., & Joo, S.-T. (2022). Vascular rinsing and chilling carcasses improves meat quality and food safety: A Review. *Journal of Animal Science and Technology*, 64(3), 397–408. https://doi.org/10.5187/jast.2022.e29

Khliji, S., van de Ven, R., Lamb, T. A., Lanza, M., & Hopkins, D. L. (2010). Relationship between consumer ranking of Lamb Colour and objective measures of colour. *Meat Science*, 85(2), 224–229. https://doi.org/10.1016/j.meatsci.2010.01.002

Kim, Y. H., Warner, R. D., & Rosenvold, K. (2014). Influence of high pre-rigor temperature and fast ph fall on muscle proteins and meat quality: A Review. *Animal Production Science*, 54(4), 375–395. https://doi.org/10.1071/an13329

Koohmaraie, M., Shackelford, S. D. (1991). Effect of calcium chloride infusion on the tenderness of lambs fed a beta-adrenergic agonist. *Journal of Animal Science*, 69(6), 2463. https://doi.org/10.2527/1991.6962463x

Koohmaraie, M., Crouse, J. D., & Mersmann, H. J. (1989). Acceleration of postmortem tenderization in ovine carcasses through infusion of calcium chloride: Effect of concentration and Ionic strength. *Journal of Animal Science*, 67(4), 934–942. https://doi.org/10.2527/jas1989.674934x

Lin, K. C., Cross, H. R., Johnson, H. K., Breidenstein, B. C., Randecker, V., & Field, R. A. (1988). Mineral composition of lamb carcasses from the United States and New Zealand. Meat Science, 24(1), 47–59. https://doi.org/10.1016/0309-1740(89)90006-5

Mickelson, M. A., & Claus, J. R. (2020). Carcass chilling method effects on color and tenderness of bison meat. *Meat Science*, *161*, 108002. https://doi.org/10.1016/j.meatsci.2019.108002

Mickelson, M. A., Warner, R. D., Polkinghorne, R. J., Seman, D. L., Crump, P. M., & Claus, J. R. (2025). Carcass chilling method and electrical stimulation effects on meat quality and color in lamb. *Meat Science*, *219*, 109662. https://doi.org/10.1016/j.meatsci.2024.109662

Offer, G., Knight, P., Jeacocke, R., Almond, R., Cousins, T., & Elsey, J. (1989). The Structural Basis of the Water-Holding, Appearance and Toughness of Meat and Meat Products. *Food Structure*, *8*(1), 151–170.

Pearson, A. M., & Dutson, T. R. (2012). *Advances in meat research, volume 1: Electrical stimulation*. Springer Netherlands.

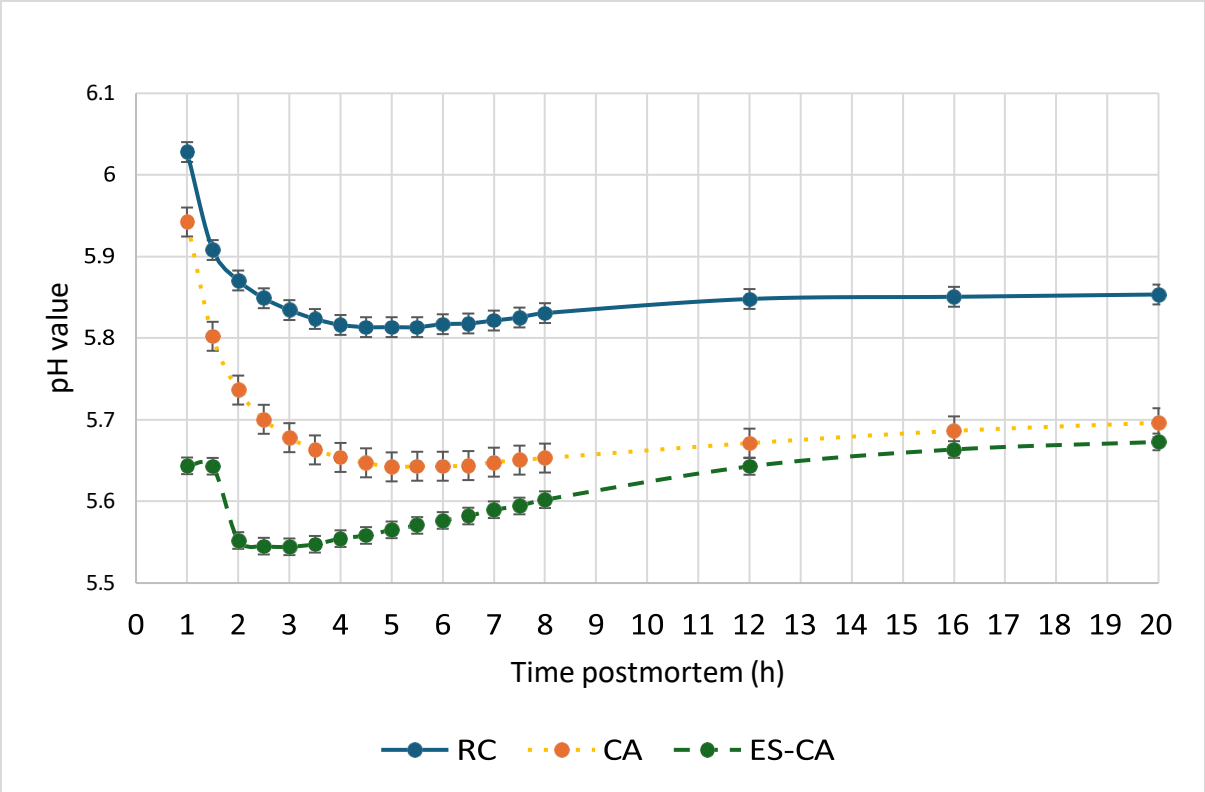
Pharino, N., Franzen, A. J., Hwang, K., Campbell, R. E., & Claus, J. R. (2024). *Vascularly rinsing lamb carcasses with calcium chloride in combination with electrical stimulation causes proteolytic changes associated with meat tenderness* (thesis).

Scheffler, T. L., & Gerrard, D. E. (2007). Mechanisms controlling pork quality development: The biochemistry controlling postmortem energy metabolism. *Meat Science*, *77*(1), 7–16. https://doi.org/10.1016/j.meatsci.2007.04.024

USDA. (2019, April 4). FoodData Central Food Details. FoodData Central. https://fdc.nal.usda.gov/fdc-app.html#/food-details/175264/nutrients

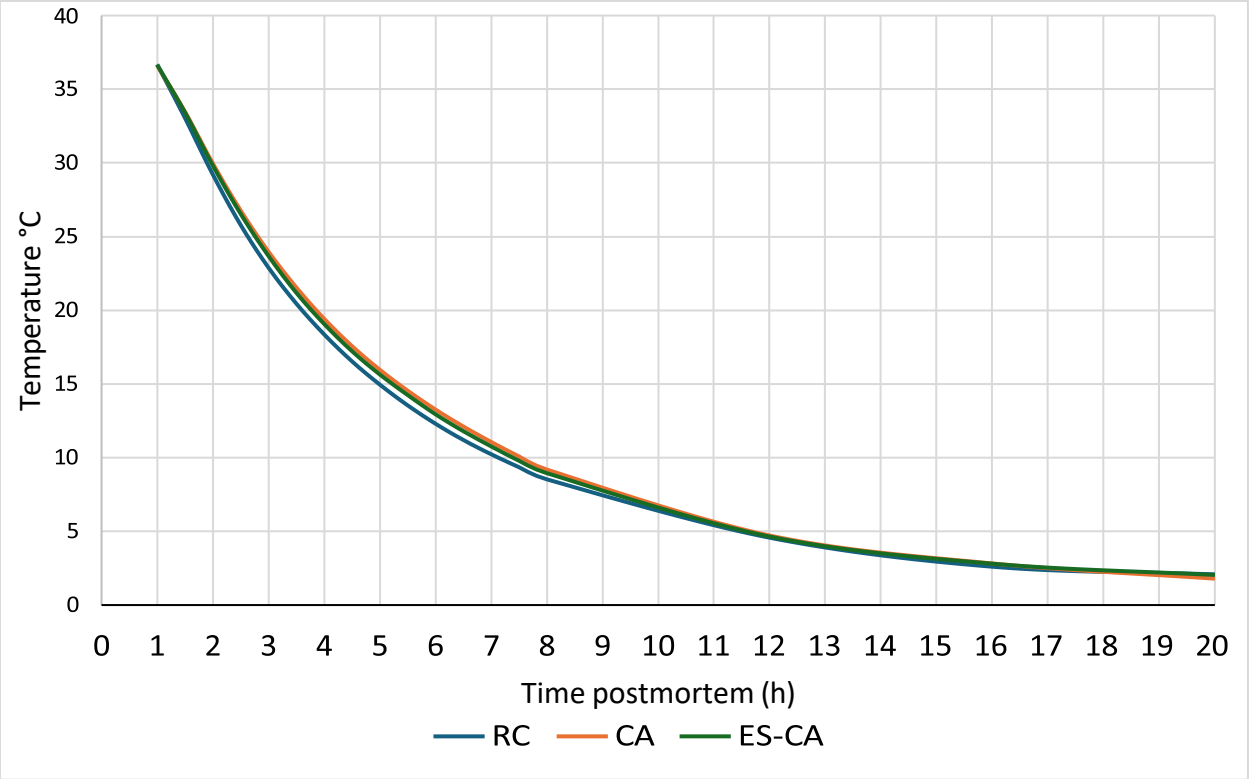
Wicks, J. C., Bodmer, J. S., Yen, C. N., Zumbaugh, M. D., Matarneh, S. K., Scheffler, T. L., Silva, S. L., Shi, H., & Gerrard, D. E. (2022). Postmortem muscle metabolism and meat quality. New Aspects of Meat Quality, 67–93. https://doi.org/10.1016/b978-0-323-85879-3.00006-

Figure 1. Postmortem pH decline (Least Square Means) in the lamb *Semimembranosus* as affected by carcass treatment.



Carcass treatment: RC = Rinse & Chill®, CA = $CaCl_2$ + RC, ES-CA = Electrical Stimulation + CA.

Figure 2. Postmortem temperature decline (Least Square Means) in the lamb *Semimembranosus* as affected by carcass treatment.



Carcass treatment: RC = Rinse & Chill®, CA = $CaCl_2$ + RC, ES-CA = Electrical Stimulation + CA.

Table 1. Least square means of lamb chops from vascularly rinsed carcasses on calcium and phosphorus content.

	Calcium (mg/kg)				Phosphorus (mg/kg)			TRT
Treatment	LD	TB	SM	TRT mean	LD	SM	TB	mean
RC	50.64^{e}	50.19^{e}	53.36^{e}	51.40	1829.93	1935.83	1761.22	1842.32
CA	699.44^{ab}	247.80^{de}	421.42^{cd}	456.22	1852.20	1979.48	1697.98	1843.22
ES-CA	915.80^{a}	414.99^{cd}	541.94^{bc}	624.24	1886.45	1958.68	1851.50	1898.88
Muscle mean	555.29	237.66	338.907		1856.19^{b}	1958.00^{a}	1770.23^{c}	

$^{a\text{-}e}$ Means with unlike letters within treatment and muscles are different ($P<0.05$). Calcium ($P<0.05$, TRT * MUSCLE, S.E.= 102.71). Phosphorus ($P<0.05$, MUSCLE= 28.33) Carcass treatment: RC = Rinse & Chill®, CA = $CaCl_2$ + RC, ES-CA = Electrical Stimulation + CA. Muscles: LD = *Longissimus thoracis et lumborum*, SM = *Semimembranosus,* TB = *Triceps brachii*

Table 2. Least square means of displayed oxygen permeable wrapped lamb chops from vascularly rinsed carcasses on instrumental color.

Dependent Variable	Treatment		
	RC	CA	ES-CA
CIE L*	42.5[a]	40.6[b]	40.2[b]
CIE a*	11.4[a]	10.0[b]	10.1[b]
Hue	48.0[a]	47.5[a]	47.8[a]
Chroma C	17.1[a]	14.8[b]	14.9[b]
OMb (%)	55.8[a]	52.2[b]	53.4[b]
DMb (%)	11.2[a]	10.2[a]	9.5[a]
MMb (%)	33.0[b]	37.5[a]	37.8[a]

[a-b]Means within a dependent variable with unlike letters are different ($P<0.05$). Treatment main effects. CIE L* (S.E.= 0.34), CIE a* (S.E. = 0.17), Hue (S.E.= 0.60), Chroma C (S.E.= 0.22), OMb (S.E.= 0.86), DMb (S.E.= 0.82), MMb (S.E.= 1.17). Carcass treatment: RC = Rinse & Chill®, CA = $CaCl_2$ + RC, ES-CA = Electrical Stimulation + CA.

Table 3: Least square means of displayed oxygen permeable wrapped lamb chops from vascularly rinsed carcasses on instrumental color.

Dependent Variable	Day	Muscle		
		LD	SM	TB
CIE L*		41.9[a]	38.9[b]	42.5[a]
CIE a*	3	10.6[b]	11.9[a]	12.0[a]
	7	8.4[c]	9.0[c]	11.0[b]
Hue	3	47.5[c]	43.3[d]	45.9[c]
	7	52.4[a]	49.9[b]	47.9[c]
Chroma C	3	15.7[c]	16.4[b]	17.3[a]
	7	13.9[d]	14.1[d]	16.2[bc]
OMb (%)	3	54.7[b]	55.6[b]	60.5[a]
	7	45.4[c]	47.9[c]	57.3[b]
DMb (%)		10.7[b]	7.8[b]	12.3[a]
MMb (%)	3	33.7[bc]	35.0[b]	25.8[d]
	7	45.6[a]	45.6[a]	31.8[c]

[a-b]Means within a dependent variable with unlike letters are different ($P<0.05$). Muscle main effects. CIE L* (S.E.= 0.34), DMb (S.E.= 0.82). [a-d]Means with unlike letters are different ($P<0.05$) Muscle x Day interactions. CIE a* (S.E.= 0.23), Hue (S.E.= 0.74), Chroma C (S.E.= 0.27), OMb (S.E.= 1.03), MMb (S.E.= 1.33). Muscles: LD = *Longissimus thoracis et lumborum*, SM = *Semimembranosus,* TB = *Triceps brachii*

Table 4: Least square means of lamb chops from vascularly rinsed carcasses on percentage purge and cook loss.

TRT and Muscle	Purge (%)			Cook Loss (%)			
	LD	SM	TB	LD	SM	TB	TRT
RC	2.1^{cd}	2.5^{cd}	1.1^{e}	24.0	22.4	19.0	21.8^{b}
CA	4.9^{b}	5.5^{ab}	1.8^{de}	25.8	22.2	21.3	23.1^{ab}
ES-CA	6.1^{a}	5.4^{ab}	2.9^{c}	28.8	23.2	22.2	24.7^{a}
Muscle	4.4	4.5	2	26.2^{a}	22.6^{b}	20.8^{b}	

$^{a\text{-}e}$Means for TRT x Muscle with unlike letters are different (interaction effects, $P<0.05$, S.E.= 0.32). $^{a\text{-}b}$Means within a dependent variable with unlike letters are different ($P<0.05$). Muscle main effects. Purge (S.E.= 0.20) Treatment main effects. Cook Loss (S.E.=0.76) Carcass treatment: RC = Rinse & Chill®, CA = $CaCl_2$ + RC, ES-CA = Electrical Stimulation + CA. Muscles: LD = *Longissimus thoracis et lumborum*, SM = *Semimembranosus,* TB = *Triceps brachii*

Table 5: Least square means of cooked lamb chops from vascularly rinsed carcasses on Warner-Bratzler Shear (newtons).

	Muscle			Day	
TRT	LD	SM	TB	3	7
RC	29.6[b]	36.5[a]	27.4[b]	35.7[a]	26.7[b]
CA	22.3[c]	34.6[a]	29.7[b]	28.8[b]	29.0[b]
ES-CA	20.9[c]	30.1[b]	29.3[b]	26.8[b]	26.8[b]

[a-c] Means with unlike letters within treatment and muscles are different ($P<0.05$, TRT*Muscle interaction, S.E.= 1.452). [a-b]Means within treatment and day with unlike letters are different ($P<0.05$, TRT*Day, S.E.= 1.229) Day = days postmortem aged before cooked (68.33°C temperature removed from oven). Carcass treatment: RC = Rinse & Chill®, CA = $CaCl_2$ + RC, ES-CA = Electrical Stimulation + CA. Muscles: LD = *Longissimus thoracis et lumborum*, SM = *Semimembranosus,* TB = *Triceps brachii*

Chapter 4

Conclusion of Thesis

This study underscores the significant advantages of vascularly delivering calcium chloride throughout the carcass to enhance meat quality. The observed reductions in shear force, particularly in the longissimus dorsi muscle, indicate that vascular rinsing with calcium is a promising method for improving tenderness. The findings offer substantial implications for the meat industry, suggesting that calcium rinsing could be an effective technique for enhancing meat quality. However, while the results are encouraging, they also highlight several areas for future research.

One critical area for further investigation is the application of calcium rinsing in beef carcasses. Given that beef generally has more tenderness issues compared to lamb, which is typically very tender, studying the effects of calcium rinsing on beef could yield substantial improvements in meat quality. Additionally, it would be valuable to explore whether rapid chilling could mitigate any color issues caused by the calcium rinse's stimulation of postmortem metabolism. This could help optimize the process, ensuring that the benefits of enhanced tenderness do not come at the cost of compromised meat color.

Moreover, future research should consider the impact of calcium rinsing on consumer acceptance. Understanding these aspects could help develop comprehensive strategies for using calcium rinsing to improve meat quality in a way that is both effective and acceptable to consumers and industry stakeholders.

In summary, the vascular delivery of calcium chloride presents a promising avenue for enhancing meat quality, particularly in terms of tenderness. However, further research is needed

to fully understand its implications and optimize its application across different types of meat. By addressing these areas, we can potentially develop more effective methods for producing high-quality meat that meets the evolving demands of the industry and consumers.