BEEF HOT CARCASS WEIGHT INFLUENCES PROTEOLYSIS

BY

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THESIS ACCEPTANCE PAGE

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABSTRACT

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The objective of this thesis was to determine the influence of beef hot carcass weight on proteolysis to better understand early postmortem proteolysis. Beef tenderness formation is influenced by various factors, such as temperature decline and proteolysis. Limited focus has been given to the effects of carcass weight on proteolysis. This study aimed to investigate the influence of increased beef hot carcass weight (HCW) on proteolysis of four muscles (*serratus ventralis*, *longissimus thoracis*, *longissimus lumborum*, and *semitendinosus*) during early postmortem aging times. Beef steers (n =12) were harvested and categorized into two weight groups: lightweight (LW; HCW = 349 ± 7.6 kg) or heavyweight (HW; HCW = 450 ± 7.6 kg). Samples were collected at nine timepoints (ten for the *longissimus lumborum*) and analyzed for disappearance of intact desmin and intact troponin-T (TnT) via Western blotting. No significant weight group x aging time interactions were observed ($P > 0.05$). An aging effect was observed on intact desmin and intact TnT in all 4 muscles ($P < 0.01$). A weight group effect on intact desmin was observed in the *serratus ventralis* ($P = 0.0203$) and *longissimus thoracis* ($P = 0.0205$) as the HW group had an increased ratio of intact desmin. A weight group effect on intact TnT was observed in the *semitendinosus* ($P = 0.0001$) as the LW group had a higher ratio of intact TnT. Results suggest increased HCW may impact proteolysis differently depending on which protein is analyzed. This study highlights the complexity

of postmortem tenderness formation and further research should be conducted to determine the effects that HCW may have on proteolysis early postmortem.

CHAPTER I: Review of Literature

Introduction

Carcass characteristics of beef cattle influence the quality of the final product and often play a role in consumers' overall acceptance of the beef. These characteristics include, but are not limited to, body weight, hot carcass weight, temperature decline, rate and extent of pH decline, and others such as Yield and Quality grades. Under the right circumstances, these characteristics result in high quality, palatable products for consumers. Palatability in meat is a term related to consumer acceptance and is referred to as "meat eating qualities" (Blumer, 1963). Three major components of palatability are flavor, tenderness, and juiciness. These three components, along with nutrition and value, often drive the demand for beef consumption as consumers desire a consistent product that meets their standards. There are many factors that influence palatability, especially when observing all three components. Often, a change in one component can affect the overall eating experience. For example, if the flavor and juiciness are acceptable, but the tenderness is not, consumer acceptance of the product will decrease. According to a consumer survey, tenderness is considered the most important palatability trait in steaks and roasts (Hunt et al., 2014). Therefore, consistency of tenderness is important to overall acceptance of beef (Becker, 2000; Mennecke et al., 2007; Moeller et al., 2010).

Tenderness can be described as how easily meat breaks down while chewing (Lorenzen et al., 2003). There are several ways to objectively and subjectively measure tenderness, such as Warner-Bratzer Shear Force (WBSF), star-probe, Sliced Shear Force (SSF), and sensory panels. Tenderness can also be predicted by measuring proteolysis of

the proteins in the meat (Melody et al., 2004). Proteolysis is the degradation or breakdown of proteins in the muscle. Many factors influence the rate and extent of proteolysis postmortem such as temperature and pH decline. One variable with historically limited research that could impact tenderness is carcass size. Average hot carcass weight has increased by over 70 kg during the last 30 years (USDA-ERS, 2024). This review will discuss the potential ways that an increase in carcass size could affect proteolysis in beef and the current understanding of early postmortem proteolysis trends in different muscles.

Increased Hot Carcass Weight Trends

Cattle producers are raising larger animals as an attempt to improve efficiency and profitability (USDA-ERS, 2024). Many processing plants have slowly increased weight limits for discounts of heavyweight carcasses, removing the signal for producers to market their animals at a lighter weight. There have also been increases in the carcass weight thresholds for the branded beef programs, further encouraging producers to sell these breeds of livestock at heavier weights (Certified Beef Programs, 2024). By increasing the carcass size, plants can get more pounds of beef per foot of chain space. The increase in total weight through the plant can improve efficiency and overall profitability. In the last 30 years, substantial improvements in growth promotants, genetics, and management practices have allowed producers to raise heavier animals quickly. Furthermore, increasing carcass weights does not necessarily mean fatter carcasses. In a study comparing carcass traits between two weight groups, heavyweight carcasses (450 $\pm$ 7.6 kg) had similar fat thickness, marbling scores, and quality grades

compared to lightweight carcasses (349 ± 7.6 kg) (DeHaan, 2022). According to the 1995 National Beef Quality Audit, average fat thickness was 1.2 cm and average HCW was 340 kg, while in 2016, fat thickness averaged 1.4 cm and average HCW was 390 kg. However, carcass size could still negatively impact meat quality. Crowding carcasses in a cooler can cause shrinkage and moisture loss due to improper chilling as elevated temperatures cause increased protein denaturation (Allen et al., 1987). Many facilities will often push carcasses as close together as possible due to the high costs of chilling systems and to maximize space, which could decrease chilling efficiency (Allen et al., 1987; Gill et al., 1991). Chilling carcasses too slowly can result in increased drip loss through protein denaturation (den Hertog-Meischke et al., 1997). Heavier carcasses chill more slowly in a commercial setting, which could lead to challenges with quality as heavy carcasses (466-523 kg) had shorter sarcomere lengths than lightweight (296-341 kg) and middle weight (386-432 kg) carcasses (Egolf, 2021).

In a study by Savell et al. (1977), when 30 sides of beef, [10 light-Good (Good was a quality grade used until Standard became the term for that grade; 75-100 kg), 10 heavy-Good (125-150 kg), and 10 heavy-Choice (125-150 kg)] were evaluated for shear force and other meat quality characteristics, the light-Good sides were statistically equivalent or superior in tenderness, cook loss, juiciness, and overall palatability in the *gluteus medius*. The light-good carcasses had significantly lower WBSF values in the *gluteus medius* compared to the two heavy groups (Savell et al., 1977). To determine the tenderness of meat, WBSF is used by measuring the amount of force required to tear through the meat. However, the *longissimus dorsi* from the light-good group had significantly greater shear force values than the heavy groups causing a contradiction that

supports studying different muscles to determine how different factors affect meat quality (Savell et al., 1977). The beef industry does not have standardization of heavyweight or lightweight as different plants have different thresholds, making comparison across studies difficult.

DeHaan (2022) observed *semitendinosus* (ST) samples from lightweight carcasses (~350 kg) were more tender (lower WBSF values) at 5 days of aging compared to heavyweight carcasses (~450 kg). However, *serratus ventralis* (SV), *longissimus thoracis* (LT), and *longissimus lumborum* (LL) WBSF values did not differ at 5, 10, 14, or 21 days of aging (DeHaan, 2022). Another study reported heavy carcasses (466-523 kg) produced lower WBSF values from the LL compared to light carcasses (296-341 kg) (Egolf, 2021). Using the same carcasses as Egolf (2021), consumers reported SV steaks from light carcasses as tougher than steaks from middle and heavy carcasses in a sensory panel (Bakker et al., 2024). Select LL steaks from middle weight carcasses were more tender than light weight and heavy weight steaks in the sensory panel (Bakker et al., 2024). Fevold et al. (2019) reported that steaks aged 14 days from different weight groups (light: < 363 kg; average: 363-408 kg; heavy: > 408 kg) did not have differences in WBSF values from the *longissimus* and the *semimembranosus*. Top round steaks, which primarily consist of the *semimembranosus*, aged 14 days also had no WBSF differences from overweight (> 432 kg) and average (341-397 kg) carcasses (Lancaster et al., 2020). The results from these studies are contradictory. This could indicate that there are factors other than carcass weight that influence the WBSF values observed in these studies. While all these studies analyzed tenderness using WBSF, there is limited research investigating the influence of carcass weight on proteolysis. More exploration is needed

to understand the effect that increased carcass weight has on beef proteolysis and tenderness.

Muscle cell structure

Skeletal muscle cells are multinucleated long cylindrical cells known as myofibers (Greaser, 1991). The functional unit of the muscle is known as the sarcomere. Sarcomeres contain thick filaments composed of the contractile protein myosin and thin filaments composed of the contractile protein actin (Greaser, 1991). The thin filament also contains the regulatory proteins tropomyosin and the troponin complex as well as the structural proteins titin and nebulin. The troponin complex consists of troponin-I, -C, and -T (TnT). Troponin-I inhibits tropomyosin from moving out of the myosin binding side on actin (Katrukha, 2013). Troponin-C binds to calcium which causes troponin-I to stop inhibition (Katrukha, 2013). Troponin-T binds to tropomyosin and connects the two proteins together (Katrukha, 2013). Titin runs from the m-line (middle of the thick filament) to the Z-disk, meaning two molecules of titin extend the length of one sarcomere (Greaser, 1991). Nebulin extends the entire length of the thin filament and together with actin help to anchor the thin filament to the Z-disk (Greaser, 1991). Other structural proteins like desmin and vinculin make up the costamere, which functions to bridge adjacent myofibrils and attach myofibrils to the sarcolemma (muscle cell membrane) (Pardo et al., 1983). Muscle cell structure plays an important role in understanding proteolysis and other mechanisms that influence tenderization in beef.

Mechanisms influencing tenderness

Tenderness is regulated by three primary mechanisms (sarcomere length, proteolysis from the calpain system, and collagen content) and can be affected by many factors such as aging time, lipid content, and species. Sarcomere length is associated with tenderness as longer sarcomeres make meat more tender (Smulders et al., 1990). Collagen content changes as animals age, with connective tissue quantity increasing while solubility decreases (Santos et al., 2016; Tuell et al., 2022). Tenderness is known to improve throughout postmortem aging out to 28 days (DeHaan, 2022; Huff-Lonergan et al., 1996). One reason for improved tenderness with aging is decreased mechanical strength of connective tissue with increased collagen solubility and dissociation of the structural integrity of muscle connective tissue during extended aging (Bakhsh et al., 2019; Nishimura, 1998; Nishimura et al., 2008). Another reason tenderness improves over time is the proteolysis of myofibrillar proteins, such as titin, nebulin, desmin, and TnT (Koohmaraie & Geesink, 2006; Taylor et al., 1995).

There have been three proteolytic systems studied in relation to tenderization in beef. The calpain system (consisting of many enzymes that are activated by calcium and their inhibitor calpastatin) is primarily responsible for myofibrillar protein degradation postmortem, leading to improved tenderness (Huff-Lonergan et al., 2010; Taylor et al., 1995; Whipple et al., 1990). Calpain-1 and calpain-2 are the two enzymes most studied in the calpain system, and they differ in how much calcium they require for activation (Cong et al., 1989; Zimmerman & Schlaepfer, 1991). Caspases are a group of enzymes that impact the calpain system's ability to degrade proteins by either increasing activation through calcium release or decreasing inhibition through calpastatin degradation (Huang

et al., 2018; Wang et al., 2018). Cathepsins are also proteolytic enzymes that degrade Z-disk proteins within a sarcomere, however, postmortem conditions do not favor cathepsin activity after the first 12 h postmortem as their activity decreases with decreased temperature (Prates et al., 2001; Taylor et al., 1995; Whipple et al., 1990).

Another factor that influences tenderness is lipid content. Previous research has shown that intramuscular lipid content has varying effects on meat tenderness depending on other factors that can alter tenderness such as pH (Lonergan et al., 2007). In a pork quality study based on pH class, lipid content was correlated to star probe value (a method to analyze the force required to tear through cooked meat) when pH was between 5.65 and 5.95 with no correlation when pH was outside that range (Lonergan et al., 2007). Lower star probe values indicate more tender meat. Therefore, as lipid content did not correlate to star probe value at all pH ranges, lipid content is a factor that can affect other mechanisms like proteolysis, which plays a larger role in tenderness changes. Species can also affect the overall tenderness of the beef as *Bos taurus* cattle are typically more tender than *Bos indicus* cattle due to differing activity levels of calpastatin (Wheeler et al., 1990; Whipple et al., 1990). Of the mechanisms of tenderness mentioned, proteolysis by the calpain system seems to be the mechanism driving improved tenderness with short-term aging (Stamler et al., 2001).

Proteolysis

During and after slaughter, a beef carcass will try to maintain homeostasis. Metabolism shifts from aerobic to anaerobic, or using oxygen to lacking oxygen (Huff-Lonergan et al., 2010). Muscle cells will continue to produce adenosine triphosphate

(ATP) using glycolysis, resulting in production of lactic acid, which lowers the pH of the carcass (Ferguson & Gerrard, 2014). The rate and extent of pH decline can influence proteolysis as calpain-1 activity is greatest at a pH of 6.5 (Maddock et al., 2005; Melody et al., 2004). pH decline is influenced by temperature decline as delayed chill (22.6°C for 6 h postmortem) carcasses have a faster rate of pH decline than conventionally chilled (2.3°C 1.25 h postmortem) carcasses (Mohrhauser et al., 2014). The rate of temperature decline also influenced proteolysis. An air temperature of 22°C resulted in increased proteolysis of isolated (in vitro) myofibrils and increased calpain activity at 72 h compared to a 4°C group despite decreased calpain activity at the elevated temperature during the first 3 h postmortem (Mohrhauser et al., 2014).

The calpain system is responsible for most of the endogenous proteolysis of meat postmortem (Taylor et al., 1995). The calpain system consists of many endogenous enzymes, with two major types responsible for degradation of skeletal muscle, known as calpain-1 and calpain-2 and their inhibitor, calpastatin. Calpastatin is the natural inhibitor of calpain and a greater concentration of calpastatin is linked to increased WBSF values (Whipple & Koohmaraie, 1992; Whipple et al., 1990). The ratio of calpastatin:calpain-1 + calpain-2 in bovine is 2.0 (Koohmaraie et al., 1991). This ratio differs depending on species and can impact proteolysis (Whipple et al., 1990). The calpains autolyze (break themselves down) under postmortem conditions (Zimmerman & Schlaepfer, 1991). The calcium requirement for activation of calpain-1 after autolysis is 5-50 μM, while calpain-2 is 200-1000 μM which is higher than typical postmortem conditions allow (Cong et al., 1989; Zimmerman & Schlaepfer, 1991). The calcium requirement for activation of

calpain-2 can be lowered by interactions with phospholipids or plasma membranes, but its speed of autolysis postmortem is evidence that it does not contribute to proteolysis differences observed during aging (Zimmerman & Schlaepfer, 1991). Calpain-2 autolysis is both intermolecular and intramolecular, meaning it will break itself down as well as neighboring molecules (Koohmaraie, 1992b). Meanwhile, calpain-1 autolysis is only intermolecular, which prevents the complete autolysis of all calpain-1 allowing prolonged proteolysis throughout the aging period (Koohmaraie, 1992b). While calpain-1 contributes to improved tenderness, calpastatin inhibits the ability of calpain-1 to perform proteolysis (Whipple et al., 1990).

Calpain-1 is a cysteine protease, a hydrolase enzyme that uses water to break proteins down into smaller pieces (Buttle & Mort, 2013). The calcium required for calpain-1 activation is released from the sarcoplasmic reticulum (Buttle & Mort, 2013). Calpain-1 is autolyzed in a postmortem system in a two-stage process from 80 kDa to an intermediate 78 kDa form, then finally to a 76 kDa subunit (Zimmerman and Schlaepfer, 1991). Also, because calpain-1 is a cysteine protease, its active site can be modified by protein S-nitrosylation affecting its autolysis and proteolytic activity (Hou et al., 2020). Autolysis of calpain-1 from 80 kDa to 76 kDa reduces the calcium requirement for calpain activation (Melody et al., 2004; Zimmerman and Schlaepfer, 1991). Once calpain-1 is autolyzed to the 76 kDa form, the 30 kDa regulatory subunit for calpain-1 is also autolyzed to an 18 kDa subunit that is important in the enhancement of calcium sensitivity for calpain-1 (Zimmerman and Schlaepfer, 1991). The reduction in calcium requirement for activation from 1 mM to 5-50 μM leads to faster rates of proteolysis immediately postmortem (Melody et al., 2004; Zimmerman and Schlaepfer, 1991). The

rate of autolysis of calpain-1 can be affected by other factors postmortem and therefore, the rate of proteolysis is also affected. It has been suggested that a rapid pH decline early postmortem results in earlier degradation of myofibrillar proteins (Melody et al., 2004). Mohrhauser et al. (2014) observed that degradation of intact TnT was greater at 22°C than at 4°C. Therefore, pH and temperature decline influence the rate of proteolysis.

Apoptosis is referred to as programmed cell death (the body's way of self-regulating cells) and is responsible for the release of calcium ions from the sarcolemma during postmortem aging (Huang et al., 2011). During aging, reactive oxygen species cause oxidative stress and apoptosis (Zhang et al., 2018). Reactive oxygen species are produced when homeostasis is disrupted and in the case of beef production, slaughter disrupts homeostasis (Wang et al., 2018). Caspase-3 is an apoptotic enzyme (causes apoptosis) that plays a significant role in degradation of structural proteins like titin and nebulin early postmortem through release of calcium and regulation of the calpain system (Huang et al., 2018; Wang et al., 2018). Caspase-3 has direct proteolytic activity against calpastatin, reducing its activity and ability to prevent proteolysis (Pörn-Ares et al., 1998). Although there is limited evidence that caspase-3 and other apoptotic caspases have direct proteolytic activity resulting in tenderization, it does play a role in calpastatin inactivation, which could result in greater calpain activity (Kemp et al., 2009). Activation of caspase-3 and other apoptotic enzymes by denitrification (the conversion of nitrate ions to nitric oxide) causes myofibril fragmentation (Wu et al., 2015). This releases calcium, which activates calpain and causes muscle contraction (Wu et al., 2015). Formation of S-nitrosothiol from the thiol group of a cysteine alters the rate of calcium release as well as inhibits phosphofructokinase (an enzyme in the glycolysis pathway),

changing the rigor mortis process and affecting pH decline, two factors that influence tenderness (Liu et al., 2016; Wang et al., 2010).

Different muscles develop tenderness at different rates, suggesting that some muscles require more time postmortem for proteolysis to reach the same level of tenderness (Koohmaraie, Seideman, et al., 1988). Each muscle within the carcass is different and contains a unique composition of different muscle fibers. Recently, cuts such as the Denver steak from the *serratus ventralis* (SV) muscle have grown in popularity. Because of its recent rise in popularity, there is limited research of proteolysis in the SV as researchers have focused on other high value muscles. When comparing the WBSF values associated with different muscles, the WBSF values of the *longissimus* decreased greatly over time compared to the *psoas major* and the *biceps femoris* (Koohmaraie, Seideman, et al., 1988). In the *longissimus* and ST, the myofibril fragmentation index (MFI) increased significantly more than in the *psoas major* (Olson & Stromer, 1976). The MFI quantifies the fragments given off the myofibril during postmortem aging, with a larger value indicating more degradation of the myofibril. Olson and Stromer (1976) also reported WBSF values decreased significantly more over time in the *longissimus* and ST than in the *psoas major*. One reason for the difference in early postmortem proteolysis rates is the calpastatin level in the muscle. Of the three muscle fiber types (type I, type IIa, and type IIb), type I muscle fibers (oxidative; red muscle fibers) have more calpastatin compared to glycolytic (white) fiber types (Koohmaraie, Seideman, et al., 1988; Olson & Stromer, 1976). Muscles like the *longissimus dorsi*, ST, and *biceps femoris* are examples of muscle with primarily white muscle fibers, while the *psoas major* (PM) contains predominantly red muscle fibers

(Christensen et al., 2004). Melody et al. (2004) observed that *semimembranosus* (SM) muscle contained significantly more calpastatin compared to the LD as well as significantly higher shear force values. *Bos indicus* cattle have greater concentrations of calpastatin and tougher meat than *Bos taurus* animals (Whipple et al., 1990). Cattle with greater concentrations of calpastatin experience decreased proteolysis of proteins linked to tenderness formation (Whipple et al., 1990).

Early Postmortem Proteolysis

In recent years, limited research on proteolysis occurring before 24 h postmortem has been conducted. Most research prior to 24 h postmortem focuses solely on calpain-1 and calpain-2 autolysis and their activity (Cong et al., 1989; Koohmaraie, 1992a). Calpain-1 can autolyze very rapidly postmortem if calcium concentrations are 200 μM (Cong et al., 1989). Once autolysis of calpain-1 has begun, calcium concentrations of 5 μM are enough to continue autolysis with maximum proteolytic activity 60 seconds after incubation *in vitro* (Cong et al., 1989). Because calpain-1 is only autolyzed intermolecularly, proteolytic activity can continue during the aging period (Koohmaraie, 1992a). While calpain-2 activity reached 0% at 9 h postmortem, calpain-1 activity remained at roughly 20% at 24 h postmortem (Koohmaraie, 1992a). As autolysis of calpain-1 slows, it is still capable of degrading other myofibrillar proteins, evidenced by disappearance of intact bands of TnT and desmin even 28 days postmortem (Huff-Lonergan et al., 1996). Studies that have analyzed degradation of proteins like desmin and TnT typically only involve samples collected just after exsanguination or after 24 h postmortem (Huff-Lonergan et al., 1996; Taylor et al., 1995). Calpain-1 activity is

highest at death and decreases throughout the aging period, so it is possible that changes to desmin and TnT occur within the first 24 h postmortem. It is understood that 65-80% of tenderness improvement occurs within the first three or four days postmortem, yet there are few studies that have investigated the degradation of myofibrillar proteins within that time frame (Taylor et al., 1995).

One of the reasons for limited research within the first 24 h postmortem is the difficulty of obtaining samples during that time. Another reason is that muscle ATP concentration is high enough to support contraction until 12-24 h postmortem, so any attempt to cook steaks and analyze tenderness via WBSF results in severe contraction (Goll et al., 1997). Severe contraction causes high shear values and shortening of the muscle during cooking (Goll et al., 1997). There were several methods used to determine tenderness during the first 24 h postmortem. The Dransfield model is an equation that assumes calpain-1 retains 24-28% of its maximal activity when meat is stored at 5°C and pH 5.5. The equation:

$$T_t = T_o e^{-kt}$$

where T_t is tenderness at some time postmortem, T_o is at-death tenderness, k is a rate constant, and t is time, is based on measurements of bovine skeletal muscle tenderness and calpain-1 activity obtained at different times postmortem (Dransfield, 1992). When calpain-1 activity was measured to determine how much activity it retained after autolysis, it was conducted at 25°C and pH 7.5 (Koohmaraie et al., 1986). Later research determined that lowering the pH to 5.5 while maintaining a temperature of 25°C reduces calpain-1 activity to 14-18% of its maximal activity (Edmunds et al., 1991). It seems unlikely that calpain-1 activity would retain 14-18% of its maximal activity once

temperatures are lowered to 4-5°C, which is the temperature of most cooling systems for meat aging. Another assumption of the Dransfield model is that calpastatin loses much of its ability to inhibit calpain-1 below pH 6.5 and is lost completely below pH 5.7 (Cottin et al., 1981). However, later research determined that pH has no effect on calpastatin's ability to inhibit calpain-1 above pH of 6.18 (Otsuka & Goll, 1987). Because calpains precipitate from aqueous solutions below pH 6.2, measuring the ability of calpastatin to inhibit calpain-1 is difficult below pH 6.2 (Goll et al., 1997). The adjustments needed to correct for these two assumptions are unclear and the Dransfield model is not a perfect method for predicting early postmortem tenderization.

A second model, referred to as the unclamped model, attempted to eliminate the effects of ATP shortening by clamping the muscle to prevent shortening during sample collection of lamb *longissimus* muscle (Wheeler & Koohmaraie, 1994). The clamped muscle was frozen at -30°C for 1.5 h and then stored at -5°C for 10 days to allow slow degradation of ATP, resulting in meat with limited or no ATP (Wheeler & Koohmaraie, 1994). The meat could then be cooked without the effects of ATP-induced shortening. The results indicated a large increase in shear force value (increased toughness) during the first 24 h followed by a similarly large decrease in shear force value (improved tenderness) by 72 h postmortem (Wheeler & Koohmaraie, 1994). The sarcomere length of the muscle decreased significantly during the first 24 h of storage, which accounts for the increased toughness. However, the sarcomere length increased only slightly from 24-72 h postmortem, indicating other mechanisms or factors decreased toughness during that time (Wheeler & Koohmaraie, 1994). Sarcomeres with lengths of 1.43 μm had significantly higher WBSF values than sarcomeres with lengths of 2.57 μm (Weaver et

al., 2008). The extent of early postmortem proteolysis of proteins like desmin and TnT is still unclear. Many studies have examined the MFI to determine the amount of degradation that occurs to the myofibril during the first 3 days postmortem. These studies have contradicting results. One study researching the differences between *bos taurus* and *bos indicus* tenderness observed no change in MFI from 1 to 3 days postmortem with a significant increase (more fragmentation) after 3 days postmortem (Whipple et al., 1990). Conversely, two studies reported that 61-64% of the increase in MFI during 7 or 14 days of aging happened during the first 3 days postmortem (Koohmaraie, Babiker, et al., 1988; Koohmaraie et al., 1987).

A third model was used to bypass the confounding effect caused by shortening of the muscle in the unclamped model (Koohmaraie et al., 1996). Like the unclamped model, the muscle was clamped to prevent shortening, however, the clamped muscle was allowed to "age" at 1.1°C for various periods of time before rapidly freezing at -30°C for 1.5 h and then storage for 8 days at -5°C to allow ATP degradation (Koohmaraie et al., 1996). Clamping the muscle prevented sarcomere shortening and resulted in no marked increase in shear value during the first 24 h postmortem (Koohmaraie et al., 1996). The shear value of the clamped muscle was almost constant during the first 72 h postmortem, suggesting proteolysis during the first 72 h does not contribute to the steep decline in shear values observed in the second model (Koohmaraie et al., 1996). Previous research has also indicated that degradation of proteins like titin, nebulin, desmin, and TnT is not to an extent that causes significant changes in tenderness during the first 72 h postmortem (Ho et al., 1994; Ho et al., 1996; Ho et al., 1997; Huff-Lonergan et al., 1995; Taylor et al., 1995). However, these studies did not evaluate degradation within the first 24 h to

determine what is occurring with the structural integrity of protein during that time. To fully understand the relationship between early postmortem proteolysis and tenderness, the degradation of proteins like desmin and TnT during the first 24 h must be explained.

Desmin

Desmin is a cytoskeletal protein in the costamere that extends to encircle the Z-disk portion of the sarcomere (Taylor et al., 1995). Costameres are composed of several proteins such as vinculin and desmin and function to maintain the structural integrity of the sarcolemma during muscular contraction (Peter et al., 2011). The costamere acts as a bridge between the sarcomere and the sarcolemma in striated muscle (Peter et al., 2011). The specific function of desmin is to connect adjacent myofibrils to each other and peripheral myofibrils to the sarcolemma at the Z-disk level (Granger and Lazarides, 1979; Clark et al., 2002). Desmin is quickly degraded by calpain postmortem and is related to tenderness formation via weakening of the thin filament interaction with Z-disk (Taylor et al., 1995). Degradation of desmin also causes destruction of the orderly structure of myofibrils, leading to improved tenderness (Uytterhaegen et al., 1994). Carlson et al. (2017) determined that pork loins with lower star probe values had more desmin degradation than loins with high star probe values, meaning greater degradation is associated with increased tenderness. Taylor et al. (1995) concluded that costameres are destroyed during the first three days postmortem and desmin is almost completely degraded during this period as well. Ouali (1990) determined that partial degradation of costameres can cause weakening of the sarcolemma and cause Ca^{2+} leakage into muscle cells. This increase in Ca^{2+} can contribute to the cascading effect of calpain-1 activation

due to decreased Ca^{2+} requirement for autolyzed calpain-1. Increased calpain-1 activation contributes to the degradation of desmin and the costamere early postmortem leading to tenderness formation (Melody et al., 2004; Taylor et al., 1995; Zimmerman & Schlaepfer, 1991). Desmin plays a crucial role in muscle structure and its degradation can be used to predict tenderness in proteolysis studies. There has not been research that analyzes both the rate at which desmin degrades within the first 48 h postmortem and how varying sizes of beef carcasses influences that degradation.

Intact desmin has a molecular weight of 55 kDa and degrades first into a transient 45 kDa band before appearing as a 38 kDa band (Huff-Lonergan et al., 1996). Appearance of the degraded bands first appears around 3 days postmortem (Ho et al., 1996; Huff-Lonergan et al., 1996). Disappearance of the intact band is also noticeable by 3 days postmortem (Ho et al., 1996). Both Ho et al. (1996) and Huff-Lonergan et al. (1996) analyzed samples at 0, 1, and 3 days postmortem. It may be beneficial to analyze proteolysis in between the time points previously used to gain a better understanding of how desmin degrades early postmortem. Egolf (2021) analyzed proteolysis of desmin across three weight groups: light (296-341 kg); middle (386-432 kg), and heavy (466-523 kg). Egolf (2021) reported that light and middle weight carcasses had greater desmin degradation than heavy weight carcasses in the SV, suggesting earlier postmortem aging in those weight groups. In the LL, Egolf (2021) observed a quality grade x HCW x aging day interaction reporting 5 day Select light weight carcasses had less desmin degradation product than other quality grades, weight groups, and aging days and greater desmin degradation in Low-Choice middle weight steaks aged 10 days compared to other groups. In the ST, Egolf (2021) reported increased desmin degradation in the Low-Choice

lightweight and Select middle weight groups compared to the Select light weight group. Based on these results, the effects of HCW on proteolysis of desmin should be studied further to explain their relationship.

Troponin-T

Troponin-T is a regulatory protein that functions by removing tropomyosin (the regulatory protein that blocks the myosin binding site on actin) from the actomyosin binding site. It is part of the troponin complex which consists of three subunits: troponin-I, troponin-C, and TnT. Troponin-I inhibits the ATPase activity of the actomyosin complex, troponin-C binds to calcium, and TnT interacts with tropomyosin (Katrukha, 2013). Troponin-T can be expressed in different isoforms, one for slow twitch skeletal muscle fibers, one for each type of fast twitch skeletal muscle fibers, and one for cardiac muscle fibers (Katrukha, 2013). Many studies have analyzed proteolysis of TnT as an attempt to understand its relationship with postmortem changes in tenderness. Western blots of TnT typically show two to three intact bands due to the different isoforms that exist in muscle. It has previously been stated that TnT degradation product is an estimate of calpain activity (McBride and Parrish, 1977). Troponin-T degradation can also be used to determine ultimate tenderness and it may be a direct contributor to postmortem tenderization (Huff-Lonergan et al., 2010; Taylor et al., 1995). Degradation of TnT weakens the thin filament, allowing for tenderness formation (Taylor et al., 1995; Whipple & Koohmaraie, 1992). Carlson et al. (2017) reported that low star probe pork loins showed more TnT degradation than high star probe loins. It is still unclear whether TnT degradation directly causes major tenderness differences or if it is just an indicator

of the amount of proteolysis that has occurred. Regardless, previous research has shown that analyzing TnT degradation is an effective way to make tenderness predictions (Carlson et al., 2017; Huff-Lonergan et al., 2010; Taylor et al., 1995). Due to its ability to determine tenderness and its effects on sarcomere structure, TnT degradation is often measured in proteolysis studies.

Intact TnT has a molecular weight of 37 kDa and degrades into a 30 kDa degradation band and 27-30 kDa fragments (Carlson et al., 2017). Appearance of the 30 kDa band has been correlated to meat that is more tender than meat without the band (Carlson et al., 2017; Huff-Lonergan et al., 1996; Huff-Lonergan et al., 2010; Macbride & Parrish Jr, 1977). Like desmin, studies that analyze TnT degradation typically collect samples at 0, 1, and 3 days postmortem and did not study the effects that HCW can have on TnT degradation (Ho et al., 1996; Ho et al., 1997; Huff-Lonergan et al., 1996). Egolf (2021) reported Low-Choice heavy weight carcasses had greater TnT degradation in the SV compared to steaks from Select and Low-Choice light and middle weight groups. Egolf (2021) observed no differences between weight groups in the LL and ST for degradation of TnT. Studying the rate and extent of TnT proteolysis between 0 and 3 days postmortem and the effects that HCW has on TnT degradation could help fill a gap in the current literature and understanding.

Muscle Differences

The *serratus ventralis* (SV), *longissimus thoracis* (LT), *longissimus lumborum* (LL), and the ST are four muscles that will be discussed at length. The SV is in the chuck and can be marketed as a Denver steak, and as part of the inside chuck roasts and

boneless short ribs. In a review of tenderness from 40 beef muscles, the SV was considered a tender (WBSF values of < 3.9 kg) muscle (Calkins & Sullivan, 2007). The SV consists of both type I and type II muscle fibers and is considered an "intermediate" (< 40% on one specific fiber type) muscle (Kirchofer et al., 2002). The LT is in the rib and is part of the ribeye while the LL is in the loin and is part of the New York strip steak. The LT and the LL are part of one large muscle known as the *longissimus dorsi*. In the tenderness review by Calkins & Sullivan (2007), the LT and LL were considered intermediate (WBSF values between 3.9 and 4.6 kg) muscles. The LT and LL consist primarily of type II muscle fibers (Christensen et al., 2004; Kirchofer et al., 2002). The ST is in the round and is known as the eye of round. The ST was considered tough in a tenderness review (Calkins & Sullivan, 2007). The ST consists of mostly type II fibers (Christensen et al., 2004; Kirchofer et al., 2002). It has been proposed that type II fibers are more tender than type I due to differing level of calpastatin (Christensen et al., 2004). However, of the four muscles discussed, the SV is consistently more tender than the LT, LL, and ST (Christensen et al., 2004; DeHaan, 2022). This is likely due to other mechanisms that influence tenderness, such as collagen content and sarcomere length. These four muscles span the length of the carcass and represent a range of typical tenderness. Selecting all four for use in a proteolysis study covers a wide range of muscle variables. There is very limited research of proteolysis of the SV. Studying proteolysis of the SV will provide the industry with new research and give a better understanding of how different muscle degrade postmortem. Usage of the SV as a high value cut has grown in popularity in recent years, increasing the interest of the industry to understand its postmortem tendencies (Calkins & Sullivan, 2007). The LT and LL have been studied

extensively due to the value associated with the rib and loin primals of beef carcasses (Ho et al., 1996; Huff-Lonergan et al., 1996). Studying proteolysis of both parts of the muscle will allow for comparison with previous research and provide insight on any differences between the anterior (LT) and posterior (LL) ends of the muscle. Proteolysis of the ST has also been studied, allowing for comparison with previous research (Hwang et al., 2004). In a study analyzing the differences in proteolysis between different temperature groups (muscle samples incubated at 5, 15, or 36 °C), the ST tended to have a faster rate of degradation compared to the *longissimus* (Hwang et al., 2004). Each muscle adds value to the study and covers a wide range of tenderness values and represents the carcass from anterior to posterior.

Muscles age at different rates, meaning they have different rates of proteolysis and changes in WBSF values postmortem. The *semimembranosus* is considered a fast aging muscle, while the *biceps femoris* would be considered slow (Taylor et al., 1995). The ST has a tendency for a faster rate of proteolysis compared to the *longissimus* (Hwang et al., 2004). A possible reason for the differences between muscles is that each muscle consists of different proportions of muscle fibers (Christensen et al., 2004; Kirchofer et al., 2002). Muscles that mostly contain type I fibers typically have higher calpastatin activity than muscles predominantly composed of type II fibers (Koohmaraie, Seideman, et al., 1988; Olson & Stromer, 1976). Analyzing the rate and extent of proteolysis in four muscles from different locations in the carcass that have different fiber type compositions could help fill a gap in the current literature.

Summary

The overall increase in average beef HCW in the United States could impact beef quality by impacting tenderness. Previous research has shown the differences in carcass chilling between heavyweight and lightweight carcasses. Carcass chilling has been known to influence many biochemical processes postmortem, such as pH decline, calpain activity (proteolysis), and sarcomere length. As some primals in heavier carcasses can take longer to cool (DeHaan, 2022; Egolf, 2021), it is possible that they will have greater amounts of proteolysis and ultimately be more tender. To better understand the influence of carcass weight on proteolysis, further research is needed.

Of the 3 mechanisms that influence tenderness postmortem, proteolysis seems to be the driver of significant change during postmortem storage. Many studies have analyzed proteolysis of desmin and TnT in different muscles at 0, 1, and 3 days postmortem, but have not assessed their degradation between these early time points. Most proteolysis work has been conducted on the *longissimus*, with limited research on smaller muscles like the SV. A study analyzing the effects of increased hot carcass weight on beef proteolysis in four muscles could help fill the current gap in literature.

LITERATURE CITED

Allen, D., Hunt, M., Filho, A. L., Danler, R., & Goll, S. (1987). Effects of spray chilling and carcass spacing on beef carcass cooler shrink and grade factors. *Journal of Animal Science, 64*(1), 165-170.

Bakhsh, A., Hwang, Y. H., & Joo, S. T. (2019). Effect of Slaughter Age on Muscle Fiber Composition, Intramuscular Connective Tissue, and Tenderness of Goat Meat during Post-Mortem Time. *Foods*, *8*(11). https://doi.org/10.3390/foods8110571

Becker, T. (2000). Consumer perception of fresh meat quality: a framework for analysis. *British Food Journal*, *102*(3), 158-176.

Buttle, D. J., & Mort, J. S. (2013). Cysteine Proteases. In W. J. Lennarz & M. D. Lane (Eds.), *Encyclopedia of Biological Chemistry (Second Edition)* (pp. 589-592). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-378630-2.00009-8

Calkins, C. R., & Sullivan, G. (2007). Ranking of beef muscles for tenderness. *University of Nebraska*, 1-5.

Carlson, K., Prusa, K., Fedler, C., Steadham, E., Outhouse, A., King, D., Huff-Lonergan, E., & Lonergan, S. (2017). Postmortem protein degradation is a key contributor to fresh pork loin tenderness. *Journal of Animal Science*, *95*(4), 1574-1586.

Certified Beef Programs. Certified Beef Programs, Agricultural Marketing Service. (2024). https://www.ams.usda.gov/grades-standards/certified-beef-programs

Christensen, M., Henckel, P., & Purslow, P. (2004). Effect of muscle type on the rate of post-mortem proteolysis in pigs. *Meat Science*, *66*(3), 595-601.

Cong, J., Goll, D., Peterson, A., & Kapprell, H. (1989). The role of autolysis in activity of the Ca2+-dependent proteinases (μ-calpain and m-calpain). *Journal of Biological Chemistry*, *264*(17), 10096-10103.

Cottin, P., Azanza, J., Vidalenc, P., Ducastaing, A., Valin, C., & Ouali, A. (1981). Characterization and purification of a Ca2+ ion-activated neutral proteinase

inhibitor in rabbit skeletal muscle. *Reproduction Nutrition Développement, 21*(2), 309-317.

DeHaan, T. C. (2022). *Influence of Beef Carcass Weight on Carcass Chilling, Steak Case Life and Quality Traits*. South Dakota State University.

den Hertog-Meischke, M., Van Laack, R., & Smulders, F. (1997). The water-holding capacity of fresh meat. *Veterinary quarterly*, *19*(4), 175-181.

Dransfield, E. (1992). Modelling post-mortem tenderisation—III: Role of calpain I in conditioning. *Meat Science*, *31*(1), 85-94.

Edmunds, T., Nagainis, P. A., Sathe, S. K., Thompson, V. F., & Goll, D. E. (1991). Comparison of the autolyzed and unautolyzed forms of μ-and m-calpain from bovine skeletal muscle. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, *1077*(2), 197-208.

Egolf, S. R. (2021). *The influence of carcass weight on beef primal temperature, tenderness, and palatability*. South Dakota State University.

Fevold, M., Grube, L., Keller, W., Carlin, K., & Maddock, R. (2019). Tenderness and Juiciness of Beef Steaks from Varying Hot Carcass Weights. *Meat and Muscle Biology*, *3*. https://doi.org/10.22175/mmb.10782

Gill, C., Jones, S., & Tong, A. (1991). Application of a temperature function integration technique to assess the hygienic adequacy of a process for spray chilling beef carcasses. *Journal of food protection*, *54*(9), 731-736.

Goll, D. E., Boehm, M. L., Geesink, G. H., & Thompson, V. F. (1997). What causes postmortem tenderization. Proc. 50th Ann. Rec. Meat Conf,

Greaser, M. L. (1991). An overview of the muscle cell cytoskeleton. Proc. Recip. Meat Conf,

Gruber, S., Tatum, J., Scanga, J., Chapman, P., Smith, G., & Belk, K. (2006). Effects of postmortem aging and USDA quality grade on Warner-Bratzler shear force values of seventeen individual beef muscles. *Journal of Animal Science*, *84*(12), 3387-3396.

Ho, C., Stromer, M., & Robson, R. (1994). Identification of the 30 kDa polypeptide in post mortem skeletal muscle as a degradation product of troponin-T. *Biochimie*, *76*(5), 369-375.

Ho, C.-Y., Stromer, M. H., & Robson, R. M. (1996). Effect of electrical stimulation on postmortem titin, nebulin, desmin, and troponin-T degradation and ultrastructural changes in bovine longissimus muscle. *Journal of Animal Science*, *74*(7), 1563-1575.

Ho, C.-Y., Stromer, M. H., Rouse, G., & Robson, R. M. (1997). Effects of electrical stimulation and postmortem storage on changes in titin, nebulin, desmin, troponin-T, and muscle ultrastructure in Bos indicus crossbred cattle. *Journal of Animal Science*, *75*(2), 366-376.

Hou, Q., Liu, R., Tian, X., & Zhang, W. (2020). Involvement of protein S-nitrosylation in regulating beef apoptosis during postmortem aging. *Food Chemistry*, *326*, 126975. https://doi.org/https://doi.org/10.1016/j.foodchem.2020.126975

Huang, F., Ding, Z., Zhang, C., Hu, H., Zhang, L., & Zhang, H. (2018). Effects of calcium and zinc ions injection on caspase-3 activation and tenderness in post-

mortem beef skeletal muscles. *International journal of food science & technology*, *53*(3), 582-589.

Huang, M., Huang, F., Xue, M., Xu, X., & Zhou, G. (2011). The effect of active caspase-3 on degradation of chicken myofibrillar proteins and structure of myofibrils. *Food Chem*, *128*(1), 22-27. https://doi.org/10.1016/j.foodchem.2011.02.062

Huff-Lonergan, E., Mitsuhashi, T., Beekman, D. D., Parrish Jr, F. C., Olson, D. G., & Robson, R. M. (1996). Proteolysis of specific muscle structural proteins by μ-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. *Journal of Animal Science*, *74*(5), 993-1008. https://core.ac.uk/reader/38934043?utm_source=linkout

Huff-Lonergan, E., Parrish Jr, F. C., & Robson, R. M. (1995). Effects of postmortem aging time, animal age, and sex on degradation of titin and nebulin in bovine longissimus muscle. *Journal of Animal Science*, *73*(4), 1064-1073.

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.

Hunt, M. R., Garmyn, A. J., O'Quinn, T. G., Corbin, C. H., Legako, J. F., Rathmann, R. J., Brooks, J. C., & Miller, M. F. (2014). Consumer assessment of beef palatability from four beef muscles from USDA Choice and Select graded carcasses. *Meat Science*, *98*(1), 1-8. https://doi.org/10.1016/j.meatsci.2014.04.004

Hwang, I., Park, B., Cho, S., & Lee, J. (2004). Effects of muscle shortening and proteolysis on Warner–Bratzler shear force in beef longissimus and semitendinosus. *Meat Science*, *68*(3), 497-505.

Katrukha, I. (2013). Human cardiac troponin complex. Structure and functions. *Biochemistry (Moscow), 78*, 1447-1465.

Kemp, C. M., King, D. A., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2009). The caspase proteolytic system in callipyge and normal lambs in longissimus, semimembranosus, and infraspinatus muscles during postmortem storage1. *Journal of Animal Science, 87*(9), 2943-2951. https://doi.org/10.2527/jas.2009-1790

Kirchofer, K. S., Calkins, C. R., & Gwartney, B. L. (2002). Fiber-type composition of muscles of the beef chuck and round1. *Journal of Animal Science, 80*(11), 2872-2878. https://doi.org/10.2527/2002.80112872x

Koohmaraie, M. (1992a). Effect of pH, temperature, and inhibitors on autolysis and catalytic activity of bovine skeletal muscle μ-calpain. *Journal of Animal Science, 70*(10), 3071-3080.

Koohmaraie, M. (1992b). Role of the neutral proteinases in postmortem muscle protein degradation and meat tenderness. Proc. Recip. Meat Conf,

Koohmaraie, M. (1994). Muscle proteinases and meat aging. *Meat Science, 36*(1-2), 93-104.

Koohmaraie, M., Babiker, A., Merkel, R., & Dutson, T. (1988). Role of Ca++-dependent proteases and lysosomal enyzmes in postmortem changes in bovine skeletal muscle. *Journal of Food Science, 53*(5), 1253-1257.

Koohmaraie, M., Doumit, M., & Wheeler, T. (1996). Meat toughening does not occur when rigor shortening is prevented. *Journal of Animal Science, 74*(12), 2935-2942.

Koohmaraie, M., & Geesink, G. H. (2006). Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Sci*, *74*(1), 34-43. https://doi.org/10.1016/j.meatsci.2006.04.025

Koohmaraie, M., Schollmeyer, J., & Dutson, T. (1986). Effect of low-calcium-requiring calcium activated factor on myofibrils under varying pH and temperature conditions. *Journal of Food Science*, *51*(1), 28-32.

Koohmaraie, M., Seideman, S., Schollmeyer, J., Dutson, T., & Babiker, A. (1988). Factors associated with the tenderness of three bovine muscles. *Journal of Food Science*, *53*(2), 407-410.

Koohmaraie, M., Seidemann, S., Schollmeyer, J., Dutson, T., & Crouse, J. (1987). Effect of post-mortem storage on Ca++-dependent proteases, their inhibitor and myofibril fragmentation. *Meat Science*, *19*(3), 187-196.

Koohmaraie, M., Whipple, G., Kretchmar, D. H., Crouse, J. D., & Mersmann, H. J. (1991). Postmortem proteolysis in longissimus muscle from beef, lamb and pork carcasses. *Journal of Animal Science*, *69*(2), 617-624. https://doi.org/10.2527/1991.692617x

Lancaster, J. M., Buseman, B. J., Weber, T. M., Nasados, J. A., Richard, R. P., Murdoch, G. K., Price, W. J., Colle, M. J., & Bass, P. D. (2020). Impact of beef carcass size on chilling rate, pH decline, display color, and tenderness of top round subprimals. *Translational animal science*, *4*(4), txaa199.

Liu, R., Li, Y., Wang, M., Zhou, G., & Zhang, W. (2016). Effect of protein S-nitrosylation on autolysis and catalytic ability of μ-calpain. *Food Chem*, *213*, 470-477. https://doi.org/10.1016/j.foodchem.2016.06.104

Lonergan, S. M., Stalder, K. J., Huff-Lonergan, E., Knight, T. J., Goodwin, R. N., Prusa, K., & Beitz, D. (2007). Influence of lipid content on pork sensory quality within pH classification. *Journal of Animal Science*, *85*(4), 1074-1079.

Lorenzen, C. L., Miller, R. K., Taylor, J. F., Neely, T. R., Tatum, J. D., Wise, J. W., Buyck, M. J., Reagan, J. O., & Savell, J. W. (2003). Beef Customer Satisfaction: Trained sensory panel ratings and Warner-Bratzler shear force values1. *Journal of Animal Science*, *81*(1), 143-149. https://doi.org/10.2527/2003.811143x

Macbride, M. A., & Parrish Jr, F. (1977). The 30,000-dalton component of tender bovine longissimus muscle. *Journal of Food Science*, *42*(6), 1627-1629.

Maddock, K., Huff-Lonergan, E., Rowe, L., & Lonergan, S. M. (2005). Effect of pH and ionic strength on μ-and m-calpain inhibition by calpastatin. *Journal of Animal Science*, *83*(6), 1370-1376.

Melody, J., Lonergan, S. M., Rowe, L., Huiatt, T. W., Mayes, M. S., & Huff-Lonergan, E. (2004). Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. *Journal of Animal Science*, *82*(4), 1195-1205.

Mennecke, B. E., Townsend, A. M., Hayes, D. J., & Lonergan, S. M. (2007). A study of the factors that influence consumer attitudes toward beef products using the conjoint market analysis tool. *Journal of Animal Science*, *85*(10), 2639-2659.

Moeller, S., Miller, R., Edwards, K., Zerby, H., Logan, K., Aldredge, T., Stahl, C., Boggess, M., & Box-Steffensmeier, J. (2010). Consumer perceptions of pork eating quality as affected by pork quality attributes and end-point cooked temperature. *Meat Science, 84*(1), 14-22.

Mohrhauser, D., Lonergan, S. M., Huff-Lonergan, E., Underwood, K., & Weaver, A. (2014). Calpain-1 activity in bovine muscle is primarily influenced by temperature, not pH decline. *Journal of Animal Science, 92*(3), 1261-1270.

Nishimura, T. (1998). Mechanism Involved in the Improvement of Meat Taste during Postmortem Aging. *Food Science and Technology International, Tokyo*, *4*(4), 241-249. https://doi.org/10.3136/fsti9596t9798.4.241

Nishimura, T., Fang, S., Ito, T., WAKAMATSU, J. i., & Takahashi, K. (2008). Structural weakening of intramuscular connective tissue during postmortem aging of pork. *Animal Science Journal*, *79*(6), 716-721.

Olson, D. G., & Stromer, M. (1976). Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. *Journal of Food Science, 41*(5), 1036-1041.

Otsuka, Y., & Goll, D. (1987). Purification of the Ca2+-dependent proteinase inhibitor from bovine cardiac muscle and its interaction with the millimolar Ca2+-dependent proteinase. *Journal of Biological Chemistry, 262*(12), 5839-5851.

Pardo, J. V., Siliciano, J., & Craig, S. W. (1983). A vinculin-containing cortical lattice in skeletal muscle: transverse lattice elements (" costameres") mark sites of attachment between myofibrils and sarcolemma. *Proceedings of the National Academy of Sciences*, *80*(4), 1008-1012.

Pörn-Ares, M. I., Samali, A., & Orrenius, S. (1998). Cleavage of the calpain inhibitor, calpastatin, during apoptosis. *Cell Death Differ*, *5*(12), 1028-1033. https://doi.org/10.1038/sj.cdd.4400424

Prates, J. A. M., Ribeiro, A. M. R., & Correia, A. A. D. (2001). Role of cysteine endopeptidases (EC 3.4. 22) in rabbit meat tenderisation and some related changes. *Meat Science*, *57*(3), 283-290.

Santos, C., Moniz, C., Roseiro, C., Tavares, M., Medeiros, V., Afonso, I., Dias, M., & Ponte, D. (2016). Effects of Early Post-Mortem Rate of pH fall and aging on Tenderness and Water Holding Capacity of Meat from Cull Dairy Holstein-Friesian Cows. *Journal of Food Research*, *5*, 1. https://doi.org/10.5539/jfr.v5n2p1

Savell, J. (2012). Beef carcass chilling: Current understanding, future challenges. *White paper Product Enhancement Research, National Cattlemen's Beef Association. Acedido em*, *29*(09), 2014.

Savell, J. W., Smith, G. C., & Carpenter, Z. L. (1977). Blade tenderization of 4 muscles from 3 weight-grade groups of beef. *Journal of Food Science*, *42*(4), 866-+. https://doi.org/10.1111/j.1365-2621.1977.tb12625.x

Sosnicki, A. A. (2015). The science of meat-An applied focus. Proc. 68th Reciprocal Meat Conference. Lincoln, NE,

Stamler, J. S., Lamas, S., & Fang, F. C. (2001). Nitrosylation. the prototypic redox-based signaling mechanism. *Cell*, *106*(6), 675-683. https://doi.org/10.1016/s0092-8674(01)00495-0

Taylor, R. G., Geesink, G. H., Thompson, V. F., Koohmaraie, M., & Goll, D. E. (1995). Is Z-disk degradation responsible for postmortem tenderization? *Journal of Animal Science*, *73*(5), 1351-1367.

Tuell, J. R., Nondorf, M. J., & Brad Kim, Y. H. (2022). Post-Harvest Strategies to Improve Tenderness of Underutilized Mature Beef: A Review. *Food Sci Anim Resour*, *42*(5), 723-743. https://doi.org/10.5851/kosfa.2022.e33

USDA-ERS - Livestock and Meat Domestic Data. (2024). https://www.ers.usda.gov/data-products/livestock-and-meat-domestic-data/

Uytterhaegen, L., Claeys, E., & Demeyer, D. (1994). Effects of exogenous protease effectors on beef tenderness development and myofibrillar degradation and solubility. *J Anim Sci*, *72*(5), 1209-1223. https://doi.org/10.2527/1994.7251209x

Wang, H., Viatchenko-Karpinski, S., Sun, J., Györke, I., Benkusky, N. A., Kohr, M. J., Valdivia, H. H., Murphy, E., Györke, S., & Ziolo, M. T. (2010). Regulation of myocyte contraction via neuronal nitric oxide synthase: role of ryanodine receptor S-nitrosylation. *J Physiol*, *588*(Pt 15), 2905-2917. https://doi.org/10.1113/jphysiol.2010.192617

Wang, L.-L., Yu, Q.-L., Han, L., Ma, X.-L., Song, R.-D., Zhao, S.-N., & Zhang, W.-H. (2018). Study on the effect of reactive oxygen species-mediated oxidative stress on the activation of mitochondrial apoptosis and the tenderness of yak meat. *Food Chemistry*, *244*, 394-402. https://doi.org/https://doi.org/10.1016/j.foodchem.2017.10.034

Weaver, A. D., Bowker, B. C., & Gerrard, D. E. (2008). Sarcomere length influences postmortem proteolysis of excised bovine semitendinosus muscle. *Journal of*

Animal Science, *86*(8), 1925-1932. https://doi.org/https://doi.org/10.2527/jas.2007-0741

Wheeler, T., & Koohmaraie, M. (1994). Prerigor and postrigor changes in tenderness of ovine longissimus muscle. *Journal of Animal Science*, *72*(5), 1232-1238.

Wheeler, T. L., Savell, J. W., Cross, H. R., Lunt, D. K., & Smith, S. B. (1990). Mechanisms associated with the variation in tenderness of meat from Brahman and Hereford cattle. *Journal of Animal Science*, *68*(12), 4206-4220. https://doi.org/10.2527/1990.68124206x

Whipple, G., & Koohmaraie, M. (1992). Effects of lamb age, muscle type, and 24-hour activity of endogenous proteinases on postmortem proteolysis. *J Anim Sci*, *70*(3), 798-804. https://doi.org/10.2527/1992.703798x

Whipple, G., Koohmaraie, M., Dikeman, M., Crouse, J., Hunt, M., & Klemm, R. (1990). Evaluation of attributes that affect longissimus muscle tenderness in Bos taurus and Bos indicus cattle. *Journal of Animal Science*, *68*(9), 2716-2728.

Wu, W., Wan, O. W., & Chung, K. K. (2015). S-nitrosylation of XIAP at Cys 213 of BIR2 domain impairs XIAP's anti-caspase 3 activity and anti-apoptotic function. *Apoptosis*, *20*(4), 491-499. https://doi.org/10.1007/s10495-015-1087-3

Zhang, J., Ma, G., Guo, Z., Yu, Q., Han, L., Han, M., & Zhu, Y. (2018). Study on the apoptosis mediated by apoptosis-inducing-factor and influencing factors of bovine muscle during postmortem aging. *Food Chem*, *266*, 359-367. https://doi.org/10.1016/j.foodchem.2018.06.032

Zimmerman, U.-J. P., & Schlaepfer, W. W. (1991). Two-stage autolysis of the catalytic subunit initiates activation of calpain I. *Biochimica et Biophysica Acta (BBA) -*

Protein Structure and Molecular Enzymology, *1078*(2), 192-198.

https://doi.org/https://doi.org/10.1016/0167-4838(91)99009-H

CHAPTER II: Beef hot carcass weight influences proteolysis

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ABSTRACT

The objective of this thesis was to determine the influence of beef hot carcass weight on proteolysis and to better understand early postmortem proteolysis. Beef tenderness formation is influenced by various factors, such as temperature decline and proteolysis. Limited focus has been given to the effects of carcass weight on proteolysis. This study aimed to investigate the influence of increased beef hot carcass weight (HCW) on proteolysis of four muscles (*serratus ventralis, longissimus thoracis, longissimus lumborum,* and *semitendinosus*) during early postmortem aging. Beef steers (n =12) were harvested and categorized into two weight groups: lightweight (LW; HCW = 349 ± 7.6 kg) or heavyweight (HW; HCW = 450 ± 7.6 kg). Samples were collected at nine timepoints (ten for the *longissimus lumborum*) and analyzed for intact desmin and intact troponin-T (TnT) disappearance via Western blotting. No significant weight group x aging time interactions were observed ($P > 0.05$). An aging effect was observed for intact desmin and intact TnT in all 4 muscles ($P < 0.01$). A weight group effect on intact desmin was observed in the *serratus ventralis* ($P = 0.0203$) and *longissimus thoracis* ($P =$

0.0205) as the HW group had an increased ratio of intact desmin. A weight group effect on intact TnT was observed in the *semitendinosus* (P = 0.0001) as the LW group had a higher ratio of intact TnT. Results suggest increased HCW may impact proteolysis differently depending on the specific protein and muscle that is analyzed. This study highlights the complexity of postmortem tenderness formation and further research should be conducted to determine the effects that HCW may have on proteolysis early postmortem.

INTRODUCTION

Beef tenderness is affected by factors such as temperature decline (DeHaan, 2022; Egolf, 2021), pH decline (Moeller et al., 2010), fiber type (Kirchofer et al., 2002), and lipid content (Lonergan et al., 2007). Temperature decline influences pH decline, calpain-1 activity, water holding capacity (WHC), and meat color (Egolf, 2021; Mohrhauser et al., 2014). Calpain-1 is a protease that degrades proteins within meat, which improves tenderness with postmortem aging (Taylor et al., 1995) . Aging time and protein degradation could be the factors mainly responsible for tenderness variation in beef. Heavy beef hot carcass weights (HCW) slow temperature decline in a large commercial setting when compared to light carcasses (Egolf, 2021). Average beef HCW has increased by over 70 kg in the last 30 years (USDA-ERS, 2024). There have been few changes to the chilling systems used in the industry during that time. Many commercial plants were designed to chill carcasses of much smaller size on average than what is

currently produced (Savell, 2012). In recent years, limited research on the effects of HCW on proteolysis has been conducted.

Proteolysis is the degradation of the primary structure of proteins. During postmortem aging, proteolysis caused by the calpain system occurs more rapidly than it does antemortem due to a reduction in the calcium requirement for calpain activation (Zimmerman & Schlaepfer, 1991). Proteolysis can be used to predict tenderness as increased protein degradation has been linked to more tender meat (Carlson et al., 2017; Taylor et al., 1995). It is well understood that meat gets more tender as it ages for up to 28 d (Huff-Lonergan et al., 1996). In recent years, new cuts such as the Denver cut from the *serratus ventralis* (SV) muscle have grown in popularity. There is limited historical research on proteolysis of muscles like the SV and *semitendinosus* (ST). Furthermore, limited research has been done on early postmortem proteolysis in recent years despite the genetic and HCW changes observed in the industry. Given the existing literature surrounding early postmortem proteolysis and the possible effects that increased HCW could have on proteolysis, it is hypothesized that heavier carcasses will experience decreased rates of proteolysis. The basis for the hypothesis is that heavier carcasses may have increased calpastatin activity (the inhibitor of calpain-1), which is part of the reason why those animals were able to grow muscle mass more quickly than the lighter animals. The purpose of this study was to determine the effects that increased beef HCW may have on proteolysis of desmin and troponin-T from four muscles and to discover any changes that may have occurred in early postmortem proteolysis.

MATERIALS AND METHODS

Sample Collection

Twelve beef steers from a single source were finished at the South Dakota State University (SDSU) Ruminant Nutrition Center and harvested at the SDSU Meat Laboratory over two slaughter days, two weeks apart. The cattle were selected based on live weight to fit within one of two weight ranges: 500 – 614 kg or 659 – 727 kg. After slaughter, carcasses were assigned to one of two weight groups based on hot carcass weight (HCW): Lightweight (LW; HCW = 349 ± 7.6 kg) or Heavyweight (HW; HCW = 450 ± 7.6 kg). Samples (at least 200 grams) for proteolysis analysis of the *longissimus lumborum* (LL) were collected at ten time points (2, 4, 6, 8, 12, 24, 48, 120, 240, and 336 h postmortem) while samples from the other three muscles, the *serratus ventralis* (SV), *longissimus thoracis* (LT), and *semitendinosus* (ST) were collected at the first nine timepoints. As the SV is located deep within the carcass, samples were collected by cutting through surrounding muscle to locate the SV. Samples were labeled, vacuum sealed, and frozen immediately at -20°C after collection. Samples were stored for two years prior to analysis.

Sample Preparation

Powdered samples were used to create protein samples for gel electrophoresis and Western Blots using methods by Melody et al. (2004) with several modifications. Briefly, a custom-made meat pulverizing apparatus made of aluminum with a bronze head resembling a mortar and pestle (interior pestle circumference: 15.4 cm, exterior mortar

circumference: 23.6 cm), was placed in liquid nitrogen. Frozen meat weighing at least 1.5 g was placed in the mortar, the pestle was placed on top and hit with a hammer until the meat was powdered. Powdered samples were stored in 7.62 x 12.7 cm sample bags (Fisher, Hanover Park, IL) at -20°C until further analysis.

After powdering, 0.60 – 0.65 g of each sample was weighed and placed into a tissue homogenizer tube with 10 ml Whole Muscle Buffer (WMB; 2% Sodium Dodecyl Sulfate [SDS], 10 mM Sodium Phosphate, pH 7.0) and homogenized using an overhead stirrer (Model RZR1; Heidolph, Schwabach, Germany) to extract myofibrillar and sarcoplasmic proteins. The sample was centrifuged at 1,500 x g for 15 minutes at 25°C. Protein concentration of the supernatant was determined in duplicate by diluting samples with a 1:20 dilution in double distilled deionized water. To determine protein concentrations, a Lowry protein assay (RC/DC Protein Assay Kit; Bio-Rad Laboratories, Hercules, CA) was used. A spectrophotometer at 750 nm wavelength (SpectraMax 190; Molecular Devices, Sunnyvale, CA) was used to analyze the protein assay. Protein concentration was determined by the lightness/darkness of the sample in each well, with higher concentrations of protein appearing darker. The associated software (SoftMax Pro 6, version 6.2.1; Molecular Devices) to the spectrophotometer evaluated protein concentrations compared to a standard curve. Protein gel samples were diluted to 6.4 mg/mL using WMB. Samples were diluted to a final concentration of 4 mg/mL by mixing 0.5 mL of gel buffer (3 mM EDTA, 3% [wt/vol] SDS, 30% [vol/vol] glycerol, 0.001% pyronin Y [wt/vol], 30 mM Tris-HCl, pH 8.0), and 0.1 mL of 2-mercaptoethanol with the sample. Samples were heated at 50°C for 15 minutes for protein denaturation and stored at -20°C.

Prior to running Western blots, load checks (visualization of the protein bands to ensure proper dilution of each sample) were performed on 15% SDS-polyacrylamide separation gels (SDS-PAGE; acrylamide: N-N'-bis-methylene acrylamide = 100:1, 0.1% SDS, 0.125% tetramethylene-diamen [TEMED], 0.075% ammonium persulfate [APS], and 0.125 M Tris HCL, pH 8.8) with 5% stacking gels (acrylamide: N-N'-bis-methylene acrylamide = 100:1, 0.1% SDS, 0.125% TEMED, 0.5% APS, and 0.125 M Tris HCl, pH 6.8). Gels were run using a mini gel electrophoresis unit (model SE-260; Hoefer Scientific, Holliston, MA) at 120 v for 380 v h. Gels were stained using Coomassie brilliant blue (40% Methanol, 7% Glacial Acetic Acid, 53% ddH_2O, 0,1% Coomassie brilliant blue R-250) for 24 h. Gels were then destained using 40% methanol and 7% glacial acetic acid for 48 h and imaged using ChemiImager 5500 (Alpha Innotech, San Leandro, CA) and Alpha EaseFC (v. 2.03; Alpha Innotech).

Western Blot Analysis

For quantification of intact desmin, a 10% SDS-PAGE gel with a 5% stacking gel was loaded with 40 mg of protein sample run at 120 v for 300 v h. For the quantification of intact troponin-T (TnT), a 15% SDS-PAGE gel was used containing 40 mg of protein sample ran at 120 v for 380 v h. Protein samples were then transferred from the gels to a polyvinylidene difluoride (PVDF; Immobilon – P; Merck Millipore, Darmstadt, Germany) membrane with a pore size of 0.45 nm using a TE-22 transfer unit (Hoefer Scientific, Holliston, Massachusetts) at 135 v h. The membrane was immersed in transfer buffer (24 mM Tris, 186 mM Glycine, and 15% Methanol) at 4°C using a refrigerated water bath (IsoTemp, model 6200 R28; Thermo Fisher Scientific, Asheville, NC). Upon

transfer completion, membranes were blocked in a 0.5% non-fat dry milk solution for 1 h. Membranes were incubated overnight in the primary antibody (1:40,000 polyclonal rabbit anti-desmin for desmin, 1:80,000 JLT-12 goat anti mouse for TnT; Iowa State University, courtesy of the Lonergan Lab, Iowa State University) at 4°C. The following day, membranes were allowed to return to room temperature for one h and then washed with PBS-Tween (66mM Sodium Phosphate, 0.1 M NaCl, and 0.1% Tween-20) three times for ten minutes per wash. After the third wash, a secondary antibody was applied to each membrane (1:20,000 goat anti-rabbit horseradish peroxidase for desmin; Product #31460 Thermo Fisher Scientific, Asheville, NC; 1:40,000 goat anti-mouse horseradish peroxidase for TnT; Product #31431 Thermo Fisher Scientific) and incubated for 1 h. Again, blots were washed three times with PBS-Tween for 10 min per wash. Using an ECL Prime detection kit (GE Healthcare, Lafayette, CO), membranes were developed. Densities of immunoreactive bands were quantified by densitometry using ChemiImager 5500 (Alpha Innotech, San Leandro, CA) and Alpha EaseFC (v. 2.03; Alpha Innotech). AlphaView SA software (Protein Simple; San Jose, CA) was used to allow for visualization and quantification of the disappearance of 55 kDa bands for intact desmin and 37 kDa bands for intact TnT. Both intact desmin and intact TnT were analyzed as a ratio to an internal standard across all Western blots. The internal standard consisted of a composite sample equally represented by both weight groups and time points (2-48 h) across all Western blots to control for inter gel variation. No 120, 240, or 336 h samples were included in the standard as those samples were added after the start of the study. Samples were run in duplicate and coefficient of variance (CV) values of less than 10% were acceptable.

Statistical Analysis

Statistical analysis was performed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The individual unit was each carcass. Western blot data were analyzed as repeated measures for the effect of weight group, time, and their interaction as fixed effects. Statistical significance was assumed at an alpha level of ≤ 0.05.

RESULTS

Desmin

Weight group did not interact with aging time ($P > 0.05$) for degradation of intact desmin for any of the muscles evaluated. Weight group influenced degradation of intact desmin in the SV ($P = 0.0203$) and LT ($P = 0.0205$), with the HW group having more intact desmin present than the LW group in both muscles (Figures 2.1, 2.2, respectively). Disappearance of intact desmin did not differ between weight groups in the LL ($P = 0.3644$) or the ST ($P = 0.3492$).

Disappearance of intact desmin responded to aging time ($P < 0.0001$) in all four muscles. In the SV, intact desmin was similar from 2 to 48 h and decreased ($P < 0.0001$) at 120 and 240 h compared to the earlier timepoints, with 120 and 240 h being similar (Figure 2.3). In the LL, intact desmin was similar from 2 to 8 h and then decreased subsequently ($P < 0.0001$) from 12 to 240 h compared to the earlier timepoints with 12 and 24 h being similar (Figure 2.4). In the ST, intact desmin was similar at 2 to 48 h and decreased ($P < 0.0001$) at 120 and 240 h compared to the earlier timepoints (Figure 2.5). In the LT, intact desmin was similar at 2 to 6 h and decreased gradually ($P < 0.0001$)

from 8 to 240 h compared to the earlier timepoints (Figure 2.6). Representative Western blot images for the degradation of desmin in the SV, LT, LL, and ST throughout the aging period can be observed in Figures 2.7, 2.8, 2.9, and 2.10, respectively.

Troponin-T

Weight group did not interact with aging time ($P > 0.05$) for degradation of intact TnT for any of the muscles evaluated. Weight group did not influence degradation of intact TnT in the SV ($P = 0.5428$), LL ($P = 0.3549$), or the LT ($P = 0.1532$). However, weight group influenced degradation of intact TnT in the ST ($P = 0.0001$) with the LW group having more intact TnT present than the HW group (Figure 2.11).

Disappearance of intact TnT responded to aging time in all four muscles ($P < 0.001$). In the SV, intact TnT increased from 2 to 6 h, was similar at 4, 6, 8, 24, and 48 h, increased at 12 h, then decreased ($P < 0.001$) at each time point from 48 to 240 h (Figure 2.12). In the LL, intact TnT was similar from 2 to 6 h, increased at 8 h, then decreased ($P < 0.001$) at 12 h postmortem. Intact TnT was similar from 12 to 24 h, decreased at 48 h, decreased further at 120 h, and reached the lowest ratio at 240 and 336 h postmortem (Figure 2.13). In the ST, intact TnT was similar from 2 to 48 h, then decreased ($P < 0.001$) at 120 and 240 h compared to earlier timepoints (Figure 2.14). In the LT, the ratio of intact TnT was greatest at 2 and 4 h, then decreased ($P < 0.001$) at 6 h postmortem with 4 and 8 h being similar. From 6 h to 120 h, the ratio of intact TnT was similar. At 240 h postmortem, intact TnT was decreased compared to 8 and 12 h, but similar to 6, 24, 48, and 120 h (Figure 2.15). Representative Western blot images for the degradatiom of

TnT in the SV, LT, LL, and ST can be observed in Figures 2.16, 2.17, 2.18, and 2.19, respectively

DISCUSSION

Desmin

The lack of a weight group x aging time interaction for desmin in all four muscles suggests that the rate of proteolysis is not affected by differences in HCW in these muscles. In the SV and LT, the HW group had a greater ratio of intact desmin than the LW group, suggesting that heavier carcasses limit proteolysis of desmin (Figures 2.1, 2.2, respectively). DeHaan reported that rib primals from HW carcasses had increased deep muscle temperatures (probes were placed in the LT) for the first 25 h of chilling (2022). Previous research has indicated that warmer temperatures during chilling increase the activity of calpain-1, which could influence the rate of proteolysis (Koohmaraie, 1992a; Mohrhauser et al., 2014). However, in the current study, no differences in the rate of proteolysis were detected between weight groups despite a difference in the extent of proteolysis in the SV and LT. The warmer temperatures observed in the rib primal and the limited degradation of intact desmin in the LT of the HW group differs from previous research. One possible theory is that heavier carcasses may have increased calpastatin activity, allowing the heavier animals to deposit more skeletal muscle and limiting postmortem degradation. DeHaan (2022) observed no differences in WBSF values between HW and LW groups in the SV and LT throughout the aging period. The difference observed in the extent of desmin proteolysis was not detectable with WBSF

values, illustrating how other mechanisms that contribute to tenderness, such as collagen content and sarcomere length, negate the differences observed in proteolysis.

Intact desmin degraded throughout the aging period differently in all muscles analyzed. In the SV, no detectable disappearance of intact desmin was observed in the first 48 h, suggesting limited change in tenderness during the first 48 h postmortem caused by proteolysis. These data support previous research that concluded the change in tenderness early postmortem is not due to proteolysis and provides knowledge of how proteins in the SV degrade during the first 24 h postmortem (Koohmaraie et al., 1996). Between 48 and 120 h, intact desmin degraded, illustrating a potential change in tenderness (Figure 2.3). Dehaan (2022) reported that the WBSF values in the SV decreased from 5 days to 14 days postmortem using the same samples as the current study. The SV is known to be relatively tender early postmortem with a moderately low aging response, which is the change in tenderness throughout aging (Gruber et al., 2006). The current study researched the SV to bridge a gap in existing literature and to better understand how the proteins in a muscle with a low aging response degrade during postmortem aging.

In the LL, the ratio of intact desmin was similar at 2, 4, 6, and 8 h postmortem, with 12 h decreased from 2 h but similar to 4, 6, 8, and 24 h, then experienced a reduction from 24 to 240 h (Figure 2.4). DeHaan (2022) reported that WBSF values decreased between 120 and 240 h postmortem in the LL and were similar between 240 and 336 h. Similar to the WBSF results, intact desmin degraded from 120 to 240 h and was similar between 240 and 336 h in the LL. Previous research (Carlson et al., 2017; Ho et al., 1996; Huff-Lonergan et al., 1996) has indicated desmin degradation contributes to tenderness

formation, which is also indicated by the LL results from this study and that of DeHaan (2022). As the LL consists of predominantly white muscle fibers, which tend to have decreased calpastatin activity compared to muscles with predominantly red muscle fibers, it experienced desmin degradation earlier postmortem than the SV (Kirchofer et al., 2002).

In the ST, no disappearance of intact desmin was detectable from 2 to 48 h postmortem, suggesting little to no change in tenderness attributable to proteolysis during that time (Figure 2.5). From 48 to 120 h postmortem, the ratio of intact desmin decreased, indicating a potential change in tenderness. DeHaan (2022), using the same samples as the current study, observed a decrease in WBSF value from 5 to 10 days postmortem in the ST. The current study reported the ratio of intact desmin to be similar at 120 and 240 h postmortem. As the ST consists of predominantly white muscle fibers, it was hypothesized that disappearance of intact desmin would be detectable earlier postmortem, like the LL. However, DeHaan (2022) reported that the ST was tougher than the LL, which may be a factor of limited degradation compared to the LL.

In the LT, the ratio of intact desmin was similar from 2 to 6 h and decreased at 8 h from 2 h (Figure 2.6). From 8 to 48 h, intact desmin was similar, then decreased at 120 h. At 240 h postmortem, intact desmin had the lowest ratio compared to all other time points. DeHaan (2022) observed a decrease in WBSF value from 120 to 240 h postmortem in the LT, further illustrating the relationship between proteolysis of desmin and tenderness. As the LT and the LL are different segments from the same muscle, similar results were expected and subsequently observed.

Troponin-T

The lack of a weight group x aging time interaction for TnT in all muscles analyzed suggests that the rate of proteolysis is not affected by differences in HCW. In the ST (Figure 2.11), the LW group had a higher ratio of intact TnT than the HW group, suggesting that lighter carcasses limit proteolysis of TnT. Weight group influenced desmin degradation in the SV and LT, and influenced TnT degradation in the ST. The differences observed indicate that carcass weight impacts proteolysis differently depending on the muscle and protein analyzed. DeHaan (2022) observed that deep muscle temperatures in the round (probes located in the *semimembranosus*) from HW carcasses were higher after 3 h of aging. As the ST is located near the surface of the carcass, the difference observed in deep muscle temperature may not affect proteolysis. If the deep muscle temperature did affect proteolysis similarly to what has been suggested previously, then the increased TnT degradation in the HW group supports previous research suggesting that warmer temperatures (~5°C at 6 h) early postmortem (< 24 h) increase the rate of proteolysis (Koohmaraie, 1992a; Mohrhauser et al., 2014). However, the current study reports a difference in the extent of TnT degradation, not a difference in the rate of proteolysis. Previous research has reported that degradation of TnT is an indicator of tenderness formation (Huff-Lonergan et al., 2010; Koohmaraie, 1994; Taylor et al., 1995). Using the same samples as the current study, DeHaan (2022) observed that the ST from HW carcasses had higher WBSF values than LW carcasses and elevated deep muscle temperatures in the round after 3 h. These findings contradict those reported by Mohrhauser et al. (2014) who concluded that warmer temperatures increase the rate of

proteolysis, which should also improve tenderness. The differences observed between proteolysis data and WBSF data are likely caused by other factors the influence tenderness, like collagen content and sarcomere length.

In the SV, the ratio of intact TnT was similar at 2, 4, and 8 h postmortem and increased at 12 h postmortem, which was unexpected as previous research has shown that TnT degrades throughout aging (Huff-Lonergan et al., 1996). Huff-Lonergan et al. (1996) used samples collected from the LL at death, 24 h postmortem, and 3 days postmortem, so it is possible that their results could have been similar if samples were analyzed at the same timepoints as the current study. After 12 and 24 h postmortem, the ratio of intact TnT decreased as expected (Figure 2.12). Potential causes of the unexpected outcome could be protein aggregation, or something related to rigor such as changes in solubility. During the onset phase of rigor, the troponin complex would still be functioning properly and be bound to tropomyosin (Filatov et al., 1999). After the completion phase of rigor, which occurs around 12-24 h postmortem in beef, tropomyosin is no longer blocking the myosin binding site on actin (Filatov et al., 1999). It is possible that prior to the formation of rigor, TnT is not as soluble and does not appear with as much intensity in Western blot images as TnT after rigor. Further research on early postmortem TnT proteolysis in the SV should be conducted to better understand the degradation of intact TnT.

In the LL, the ratio of intact TnT was similar from 2 to 6 h and increased at 8 h, which was unexpected for the same reasons stated previously. The ratio of intact TnT decreased from 8 to 12 h. From 24 to 240 h, the ratio of intact TnT decreased at each time point and 240 h was similar to 336 h (Figure 2.13). It is possible that the disappearance of the intact TnT band is more difficult to use as an indicator of tenderness when compared

to analyzing the appearance of the 30 kDa degradation product that has been analyzed in previous studies (Carlson et al., 2017; Huff-Lonergan et al., 2010). Carlson et al. (2017) reported a 26% difference in intact TnT, but a 100% difference in the degradation band of TnT from pork loins aged 11-16 d, illustrating how smaller changes in the disappearance of intact TnT can cause larger changes in the appearance of the degraded band. In other words, early postmortem there are no detectable degradation bands while the intact band appears intensely. After aging, degradation bands begin to appear while the intact band loses intensity. The intact band does not fully disappear until around 28 d of aging, so samples analyzed prior to 28 d will appear with some intensity (Huff-Lonergan et al., 1996). For these reasons, a small change in the intensity of the intact band (26%) can cause a large change (100%) in the degraded band (Carlson et al., 2017).

In the ST, no statistically significant differences in intact TnT were observed from 2 to 48 h postmortem, suggesting little to no change in tenderness from proteolysis during that time (Figure 2.14). From 48 to 120 h postmortem, the ratio of intact TnT decreased, indicating a potential change in tenderness. DeHaan, using the same samples as the current study, observed a decrease in WBSF value from 120 to 240 h postmortem in the ST (2022). The current study reported that the ratio of intact TnT decreased from 120 to 240 h postmortem, illustrating the relationship between proteolysis and tenderness. Similar to the results from desmin, the ST did not experience degradation until 48 h postmortem despite consisting predominantly of white muscle fibers.

In the LT, the ratio of intact TnT was similar from 2 to 4 h and decreased at 6 h. From 6 to 120 h, the ratio of intact TnT was similar. At 240 h postmortem, intact TnT was decreased compared to 8 and 12 h, but similar to 6, 24, 48, and 120 h (Figure 2.15).

These data suggest that the change in WBSF value from 120 to 240 h observed in the LT by DeHaan (2022) was caused by something other than TnT degradation, such as degradation of structural proteins like desmin. It was hypothesized that TnT degradation from the LT and the LL would be similar as they are two parts of the same muscle. However, the LL produced unexpected TnT results for reasons stated previously.

IMPLICATIONS

Results from this study suggest that beef carcass weight and muscle type impacts proteolysis of desmin and TnT differently. Heavier HCW limits the extent of desmin degradation in the SV and LT while increasing the extent of TnT degradation in the ST. These data suggest that increased beef carcass weights could influence the extent of proteolysis that occurs in aging meat, but not to a degree that would cause tenderness differences detectable by WBSF because of mechanisms like collagen content and sarcomere length. The protein degradation observed early postmortem supports the hypothesis that proteolysis is occurring immediately postmortem and continues throughout the aging period. Degradation of desmin and TnT often increases after 24 h of aging, illustrating the importance of aging meat prior to selling to consumers for product consistency. Further research, such as a similar study investigating early postmortem proteolysis differences between two groups, should be conducted to better understand the disappearance of intact TnT in the SV and LL within the first 12 h postmortem.

LITERATURE CITED

Allen, D., Hunt, M., Filho, A. L., Danler, R., & Goll, S. (1987). Effects of spray chilling and carcass spacing on beef carcass cooler shrink and grade factors. *Journal of Animal Science*, *64*(1), 165-170.

Bakhsh, A., Hwang, Y. H., & Joo, S. T. (2019). Effect of Slaughter Age on Muscle Fiber Composition, Intramuscular Connective Tissue, and Tenderness of Goat Meat during Post-Mortem Time. *Foods*, *8*(11). https://doi.org/10.3390/foods8110571

Bakker, C. E., Egolf, S. R., O'Sullivan, L. M., Cox, R. B., Rode-Atkins, H. R., Blair, A. D., Underwood, K. R., & Grubbs, J. K. (2024). Influence of Beef Hot Carcass Weight on Sensory Characteristics of Strip Loin, Eye of Round, and Denver Cut Steaks. *Foods*, *13*(6), 961.

Becker, T. (2000). Consumer perception of fresh meat quality: a framework for analysis. *British Food Journal*, *102*(3), 158-176.

Blumer, T. (1963). Relationship of marbling to the palatability of beef. *Journal of Animal Science*, *22*(3), 771-778.

Buttle, D. J., & Mort, J. S. (2013). Cysteine Proteases. In W. J. Lennarz & M. D. Lane (Eds.), *Encyclopedia of Biological Chemistry (Second Edition)* (pp. 589-592). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-378630-2.00009-8

Calkins, C. R., & Sullivan, G. (2007). Ranking of beef muscles for tenderness. *University of Nebraska*, 1-5.

Carlson, K., Prusa, K., Fedler, C., Steadham, E., Outhouse, A., King, D., Huff-Lonergan, E., & Lonergan, S. (2017). Postmortem protein degradation is a key contributor to fresh pork loin tenderness. *Journal of Animal Science*, *95*(4), 1574-1586.

Christensen, M., Henckel, P., & Purslow, P. (2004). Effect of muscle type on the rate of post-mortem proteolysis in pigs. *Meat Science*, *66*(3), 595-601.

Cong, J., Goll, D., Peterson, A., & Kapprell, H. (1989). The role of autolysis in activity of the Ca2+-dependent proteinases (μ-calpain and m-calpain). *Journal of Biological Chemistry*, *264*(17), 10096-10103.

Cottin, P., Azanza, J., Vidalenc, P., Ducastaing, A., Valin, C., & Ouali, A. (1981). Characterization and purification of a Ca2+ ion-activated neutral proteinase inhibitor in rabbit skeletal muscle. *Reproduction Nutrition Développement*, *21*(2), 309-317.

DeHaan, T. C. (2022). *Influence of Beef Carcass Weight on Carcass Chilling, Steak Case Life and Quality Traits*. South Dakota State University.

den Hertog-Meischke, M., Van Laack, R., & Smulders, F. (1997). The water-holding capacity of fresh meat. *Veterinary quarterly*, *19*(4), 175-181.

Dransfield, E. (1992). Modelling post-mortem tenderisation—III: Role of calpain I in conditioning. *Meat Science*, *31*(1), 85-94.

Edmunds, T., Nagainis, P. A., Sathe, S. K., Thompson, V. F., & Goll, D. E. (1991). Comparison of the autolyzed and unautolyzed forms of μ-and m-calpain from bovine skeletal muscle. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, *1077*(2), 197-208.

Egolf, S. R. (2021). *The influence of carcass weight on beef primal temperature, tenderness, and palatability*. South Dakota State University.

Ferguson, D., & Gerrard, D. (2014). Regulation of post-mortem glycolysis in ruminant muscle. *Animal Production Science*, *54*(4), 464-481.

Filatov, V., Katrukha, A., Bulargina, T., & Gusev, N. (1999). Troponin: structure, properties, and mechanism of functioning. *Biochemistry c/c of Biokhimiia*, *64*, 969-985.

Gill, C., Jones, S., & Tong, A. (1991). Application of a temperature function integration technique to assess the hygienic adequacy of a process for spray chilling beef carcasses. *Journal of food protection*, *54*(9), 731-736.

Goll, D. E., Boehm, M. L., Geesink, G. H., & Thompson, V. F. (1997). What causes postmortem tenderization. Proc. 50th Ann. Rec. Meat Conf,

Greaser, M. L. (1991). An overview of the muscle cell cytoskeleton. Proc. Recip. Meat Conf,

Gruber, S., Tatum, J., Scanga, J., Chapman, P., Smith, G., & Belk, K. (2006). Effects of postmortem aging and USDA quality grade on Warner-Bratzler shear force values of seventeen individual beef muscles. *Journal of Animal Science*, *84*(12), 3387-3396.

Ho, C., Stromer, M., & Robson, R. (1994). Identification of the 30 kDa polypeptide in post mortem skeletal muscle as a degradation product of troponin-T. *Biochimie*, *76*(5), 369-375.

Ho, C.-Y., Stromer, M. H., & Robson, R. M. (1996). Effect of electrical stimulation on postmortem titin, nebulin, desmin, and troponin-T degradation and ultrastructural

changes in bovine longissimus muscle. *Journal of Animal Science*, *74*(7), 1563-1575.

Ho, C.-Y., Stromer, M. H., Rouse, G., & Robson, R. M. (1997). Effects of electrical stimulation and postmortem storage on changes in titin, nebulin, desmin, troponin-T, and muscle ultrastructure in Bos indicus crossbred cattle. *Journal of Animal Science*, *75*(2), 366-376.

Hou, Q., Liu, R., Tian, X., & Zhang, W. (2020). Involvement of protein S-nitrosylation in regulating beef apoptosis during postmortem aging. *Food Chemistry*, *326*, 126975. https://doi.org/https://doi.org/10.1016/j.foodchem.2020.126975

Huang, F., Ding, Z., Zhang, C., Hu, H., Zhang, L., & Zhang, H. (2018). Effects of calcium and zinc ions injection on caspase-3 activation and tenderness in post-mortem beef skeletal muscles. *International journal of food science & technology*, *53*(3), 582-589.

Huang, M., Huang, F., Xue, M., Xu, X., & Zhou, G. (2011). The effect of active caspase-3 on degradation of chicken myofibrillar proteins and structure of myofibrils. *Food Chem*, *128*(1), 22-27. https://doi.org/10.1016/j.foodchem.2011.02.062

Huff-Lonergan, E., Mitsuhashi, T., Beekman, D. D., Parrish Jr, F. C., Olson, D. G., & Robson, R. M. (1996). Proteolysis of specific muscle structural proteins by μ-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. *Journal of Animal Science*, *74*(5), 993-1008. https://core.ac.uk/reader/38934043?utm_source=linkout

Huff-Lonergan, E., Parrish Jr, F. C., & Robson, R. M. (1995). Effects of postmortem aging time, animal age, and sex on degradation of titin and nebulin in bovine longissimus muscle. *Journal of Animal Science*, *73*(4), 1064-1073.

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.

Hunt, M. R., Garmyn, A. J., O'Quinn, T. G., Corbin, C. H., Legako, J. F., Rathmann, R. J., Brooks, J. C., & Miller, M. F. (2014). Consumer assessment of beef palatability from four beef muscles from USDA Choice and Select graded carcasses. *Meat Science*, *98*(1), 1-8. https://doi.org/10.1016/j.meatsci.2014.04.004

Hwang, I., Park, B., Cho, S., & Lee, J. (2004). Effects of muscle shortening and proteolysis on Warner–Bratzler shear force in beef longissimus and semitendinosus. *Meat Science*, *68*(3), 497-505.

Katrukha, I. (2013). Human cardiac troponin complex. Structure and functions. *Biochemistry (Moscow)*, *78*, 1447-1465.

Kemp, C. M., King, D. A., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2009). The caspase proteolytic system in callipyge and normal lambs in longissimus, semimembranosus, and infraspinatus muscles during postmortem storage1. *Journal of Animal Science*, *87*(9), 2943-2951. https://doi.org/10.2527/jas.2009-1790

Kirchofer, K. S., Calkins, C. R., & Gwartney, B. L. (2002). Fiber-type composition of muscles of the beef chuck and round1. *Journal of Animal Science*, *80*(11), 2872-2878. https://doi.org/10.2527/2002.80112872x

Koohmaraie, M. (1992a). Effect of pH, temperature, and inhibitors on autolysis and catalytic activity of bovine skeletal muscle μ-calpain. *Journal of Animal Science*, *70*(10), 3071-3080.

Koohmaraie, M. (1992b). Role of the neutral proteinases in postmortem muscle protein degradation and meat tenderness. Proc. Recip. Meat Conf,

Koohmaraie, M. (1994). Muscle proteinases and meat aging. *Meat Science*, *36*(1-2), 93-104.

Koohmaraie, M., Babiker, A., Merkel, R., & Dutson, T. (1988). Role of Ca++-dependent proteases and lysosomal enyzmes in postmortem changes in bovine skeletal muscle. *Journal of Food Science, 53*(5), 1253-1257.

Koohmaraie, M., Doumit, M., & Wheeler, T. (1996). Meat toughening does not occur when rigor shortening is prevented. *Journal of Animal Science, 74*(12), 2935-2942.

Koohmaraie, M., & Geesink, G. H. (2006). Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Sci, 74*(1), 34-43. https://doi.org/10.1016/j.meatsci.2006.04.025

Koohmaraie, M., Schollmeyer, J., & Dutson, T. (1986). Effect of low-calcium-requiring calcium activated factor on myofibrils under varying pH and temperature conditions. *Journal of Food Science, 51*(1), 28-32.

Koohmaraie, M., Seideman, S., Schollmeyer, J., Dutson, T., & Babiker, A. (1988). Factors associated with the tenderness of three bovine muscles. *Journal of Food Science, 53*(2), 407-410.

Koohmaraie, M., Seidemann, S., Schollmeyer, J., Dutson, T., & Crouse, J. (1987). Effect of post-mortem storage on Ca++-dependent proteases, their inhibitor and myofibril fragmentation. *Meat Science*, *19*(3), 187-196.

Koohmaraie, M., Whipple, G., Kretchmar, D. H., Crouse, J. D., & Mersmann, H. J. (1991). Postmortem proteolysis in longissimus muscle from beef, lamb and pork carcasses. *Journal of Animal Science*, *69*(2), 617-624. https://doi.org/10.2527/1991.692617x

Lancaster, J. M., Buseman, B. J., Weber, T. M., Nasados, J. A., Richard, R. P., Murdoch, G. K., Price, W. J., Colle, M. J., & Bass, P. D. (2020). Impact of beef carcass size on chilling rate, pH decline, display color, and tenderness of top round subprimals. *Translational animal science*, *4*(4), txaa199.

Liu, R., Li, Y., Wang, M., Zhou, G., & Zhang, W. (2016). Effect of protein S-nitrosylation on autolysis and catalytic ability of μ-calpain. *Food Chem*, *213*, 470-477. https://doi.org/10.1016/j.foodchem.2016.06.104

Lonergan, S. M., Stalder, K. J., Huff-Lonergan, E., Knight, T. J., Goodwin, R. N., Prusa, K., & Beitz, D. (2007). Influence of lipid content on pork sensory quality within pH classification. *Journal of Animal Science*, *85*(4), 1074-1079.

Lorenzen, C. L., Miller, R. K., Taylor, J. F., Neely, T. R., Tatum, J. D., Wise, J. W., Buyck, M. J., Reagan, J. O., & Savell, J. W. (2003). Beef Customer Satisfaction: Trained sensory panel ratings and Warner-Bratzler shear force values1. *Journal of Animal Science*, *81*(1), 143-149. https://doi.org/10.2527/2003.811143x

Macbride, M. A., & Parrish Jr, F. (1977). The 30,000-dalton component of tender bovine longissimus muscle. *Journal of Food Science*, *42*(6), 1627-1629.

Maddock, K., Huff-Lonergan, E., Rowe, L., & Lonergan, S. M. (2005). Effect of pH and ionic strength on μ-and m-calpain inhibition by calpastatin. *Journal of Animal Science*, *83*(6), 1370-1376.

Melody, J., Lonergan, S. M., Rowe, L., Huiatt, T. W., Mayes, M. S., & Huff-Lonergan, E. (2004). Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. *Journal of Animal Science*, *82*(4), 1195-1205.

Mennecke, B. E., Townsend, A. M., Hayes, D. J., & Lonergan, S. M. (2007). A study of the factors that influence consumer attitudes toward beef products using the conjoint market analysis tool. *Journal of Animal Science*, *85*(10), 2639-2659.

Moeller, S., Miller, R., Edwards, K., Zerby, H., Logan, K., Aldredge, T., Stahl, C., Boggess, M., & Box-Steffensmeier, J. (2010). Consumer perceptions of pork eating quality as affected by pork quality attributes and end-point cooked temperature. *Meat Science*, *84*(1), 14-22.

Mohrhauser, D., Lonergan, S. M., Huff-Lonergan, E., Underwood, K., & Weaver, A. (2014). Calpain-1 activity in bovine muscle is primarily influenced by temperature, not pH decline. *Journal of Animal Science*, *92*(3), 1261-1270.

Nishimura, T. (1998). Mechanism Involved in the Improvement of Meat Taste during Postmortem Aging. *Food Science and Technology International, Tokyo*, *4*(4), 241-249. https://doi.org/10.3136/fsti9596t9798.4.241

Nishimura, T., Fang, S., Ito, T., WAKAMATSU, J. i., & Takahashi, K. (2008). Structural weakening of intramuscular connective tissue during postmortem aging of pork. *Animal Science Journal*, *79*(6), 716-721.

Olson, D. G., & Stromer, M. (1976). Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. *Journal of Food Science*, *41*(5), 1036-1041.

Otsuka, Y., & Goll, D. (1987). Purification of the Ca2+-dependent proteinase inhibitor from bovine cardiac muscle and its interaction with the millimolar Ca2+-dependent proteinase. *Journal of Biological Chemistry*, *262*(12), 5839-5851.

Pardo, J. V., Siliciano, J., & Craig, S. W. (1983). A vinculin-containing cortical lattice in skeletal muscle: transverse lattice elements (" costameres") mark sites of attachment between myofibrils and sarcolemma. *Proceedings of the National Academy of Sciences*, *80*(4), 1008-1012.

Pörn-Ares, M. I., Samali, A., & Orrenius, S. (1998). Cleavage of the calpain inhibitor, calpastatin, during apoptosis. *Cell Death Differ*, *5*(12), 1028-1033. https://doi.org/10.1038/sj.cdd.4400424

Prates, J. A. M., Ribeiro, A. M. R., & Correia, A. A. D. (2001). Role of cysteine endopeptidases (EC 3.4. 22) in rabbit meat tenderisation and some related changes. *Meat Science*, *57*(3), 283-290.

Santos, C., Moniz, C., Roseiro, C., Tavares, M., Medeiros, V., Afonso, I., Dias, M., & Ponte, D. (2016). Effects of Early Post-Mortem Rate of pH fall and aging on Tenderness and Water Holding Capacity of Meat from Cull Dairy Holstein-Friesian Cows. *Journal of Food Research*, *5*, 1. https://doi.org/10.5539/jfr.v5n2p1

Savell, J. (2012). Beef carcass chilling: Current understanding, future challenges. *White paper Product Enhancement Research, National Cattlemen's Beef Association. Acedido em*, *29*(09), 2014.

Savell, J. W., Smith, G. C., & Carpenter, Z. L. (1977). Blade tenderization of 4 muscles from 3 weight-grade groups of beef. *Journal of Food Science*, *42*(4), 866-+. https://doi.org/10.1111/j.1365-2621.1977.tb12625.x

Smulders, F. J. M., Marsh, B. B., Swartz, D. R., Russell, R. L., & Hoenecke, M. E. (1990). Beef tenderness and sarcomere length. *Meat Science*, *28*(4), 349-363. https://doi.org/https://doi.org/10.1016/0309-1740(90)90048-B

Stamler, J. S., Lamas, S., & Fang, F. C. (2001). Nitrosylation. the prototypic redox-based signaling mechanism. *Cell*, *106*(6), 675-683. https://doi.org/10.1016/s0092-8674(01)00495-0

Taylor, R. G., Geesink, G. H., Thompson, V. F., Koohmaraie, M., & Goll, D. E. (1995). Is Z-disk degradation responsible for postmortem tenderization? *Journal of Animal Science*, *73*(5), 1351-1367.

Tuell, J. R., Nondorf, M. J., & Brad Kim, Y. H. (2022). Post-Harvest Strategies to Improve Tenderness of Underutilized Mature Beef: A Review. *Food Sci Anim Resour*, *42*(5), 723-743. https://doi.org/10.5851/kosfa.2022.e33

Uytterhaegen, L., Claeys, E., & Demeyer, D. (1994). Effects of exogenous protease effectors on beef tenderness development and myofibrillar degradation and solubility. *J Anim Sci*, *72*(5), 1209-1223. https://doi.org/10.2527/1994.7251209x

Wang, H., Viatchenko-Karpinski, S., Sun, J., Györke, I., Benkusky, N. A., Kohr, M. J., Valdivia, H. H., Murphy, E., Györke, S., & Ziolo, M. T. (2010). Regulation of myocyte contraction via neuronal nitric oxide synthase: role of ryanodine receptor S-nitrosylation. *J Physiol*, *588*(Pt 15), 2905-2917. https://doi.org/10.1113/jphysiol.2010.192617

Wang, L.-L., Yu, Q.-L., Han, L., Ma, X.-L., Song, R.-D., Zhao, S.-N., & Zhang, W.-H. (2018). Study on the effect of reactive oxygen species-mediated oxidative stress on the activation of mitochondrial apoptosis and the tenderness of yak meat. *Food Chemistry*, *244*, 394-402. https://doi.org/https://doi.org/10.1016/j.foodchem.2017.10.034

Weaver, A. D., Bowker, B. C., & Gerrard, D. E. (2008). Sarcomere length influences postmortem proteolysis of excised bovine semitendinosus muscle. *Journal of Animal Science*, *86*(8), 1925-1932. https://doi.org/https://doi.org/10.2527/jas.2007-0741

Wheeler, T., & Koohmaraie, M. (1994). Prerigor and postrigor changes in tenderness of ovine longissimus muscle. *Journal of Animal Science*, *72*(5), 1232-1238.

Wheeler, T. L., Savell, J. W., Cross, H. R., Lunt, D. K., & Smith, S. B. (1990). Mechanisms associated with the variation in tenderness of meat from Brahman and Hereford cattle. *Journal of Animal Science*, *68*(12), 4206-4220. https://doi.org/10.2527/1990.68124206x

Whipple, G., & Koohmaraie, M. (1992). Effects of lamb age, muscle type, and 24-hour activity of endogenous proteinases on postmortem proteolysis. *J Anim Sci*, *70*(3), 798-804. https://doi.org/10.2527/1992.703798x

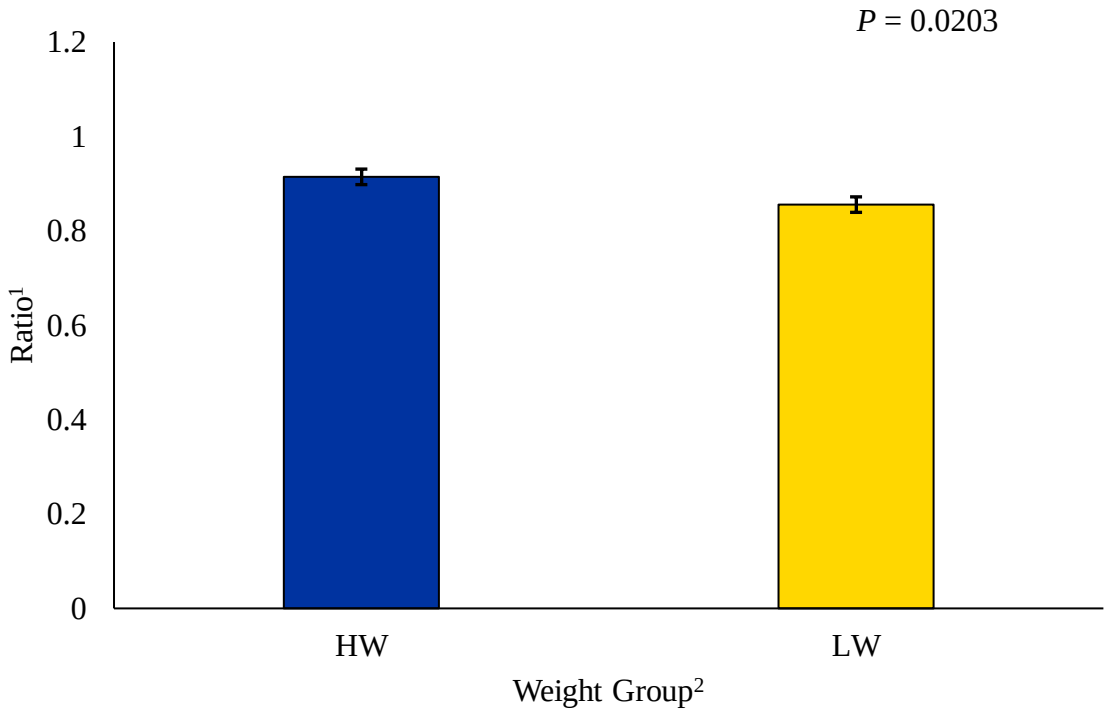
Whipple, G., Koohmaraie, M., Dikeman, M., Crouse, J., Hunt, M., & Klemm, R. (1990). Evaluation of attributes that affect longissimus muscle tenderness in Bos taurus and Bos indicus cattle. *Journal of Animal Science*, *68*(9), 2716-2728.

Wu, W., Wan, O. W., & Chung, K. K. (2015). S-nitrosylation of XIAP at Cys 213 of BIR2 domain impairs XIAP's anti-caspase 3 activity and anti-apoptotic function. *Apoptosis*, *20*(4), 491-499. https://doi.org/10.1007/s10495-015-1087-3

Zhang, J., Ma, G., Guo, Z., Yu, Q., Han, L., Han, M., & Zhu, Y. (2018). Study on the apoptosis mediated by apoptosis-inducing-factor and influencing factors of bovine muscle during postmortem aging. *Food Chem*, *266*, 359-367. https://doi.org/10.1016/j.foodchem.2018.06.032

Zimmerman, U.-J. P., & Schlaepfer, W. W. (1991). Two-stage autolysis of the catalytic subunit initiates activation of calpain I. *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology*, *1078*(2), 192-198. https://doi.org/https://doi.org/10.1016/0167-4838(91)99009-H

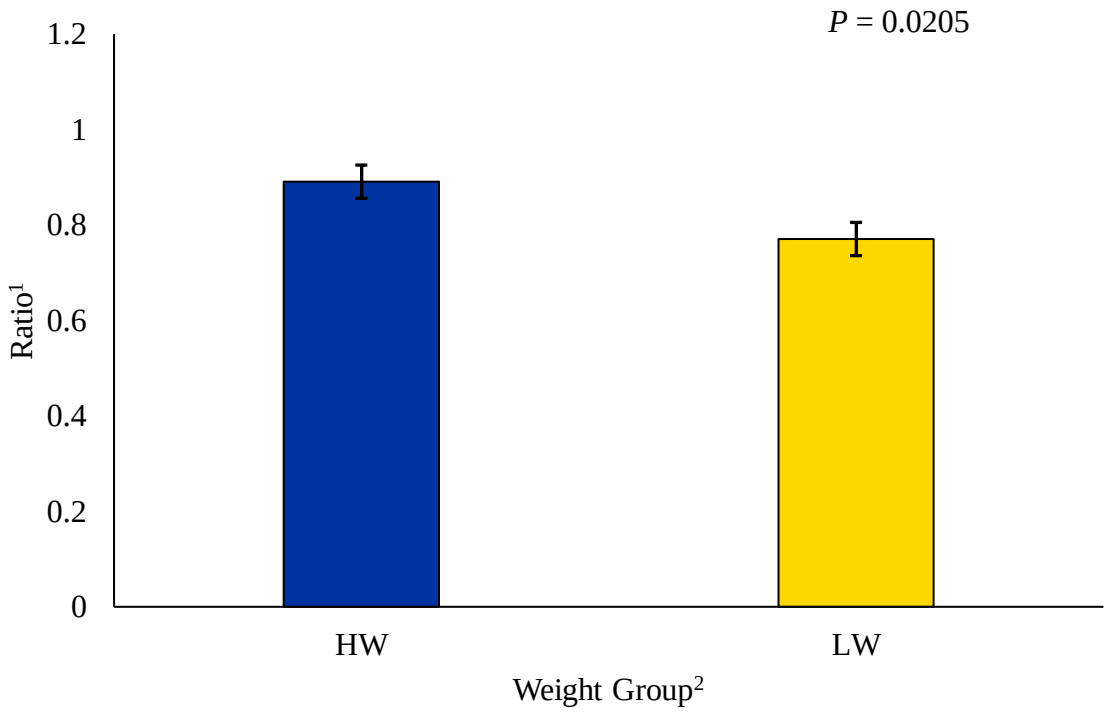
Figure 2.1 - Least square means for the effect of weight group on proteolysis of intact desmin in the *serratus ventralis* (55 kDa)



[1] Expressed as a ratio of intact desmin to an internal standard

[2] heavyweight (HW) group (450 ± 7.6 kg), lightweight (LW) group (349 ± 7.6 kg)

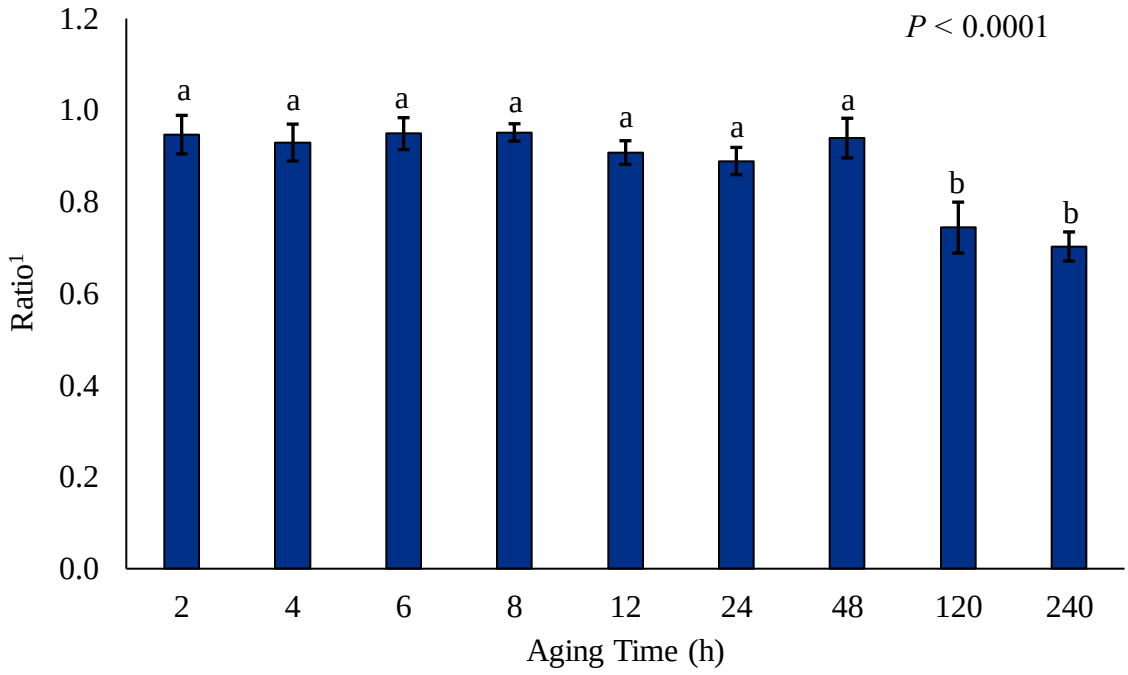
Figure 2.2 - Least square means for the effect of weight group on proteolysis of intact desmin in the *longissimus thoracis* (55 kDa)



[1] Expressed as a ratio of intact desmin to an internal standard

[2] heavyweight (HW) group (450 ± 7.6 kg), lightweight (LW) group (349 ± 7.6 kg)

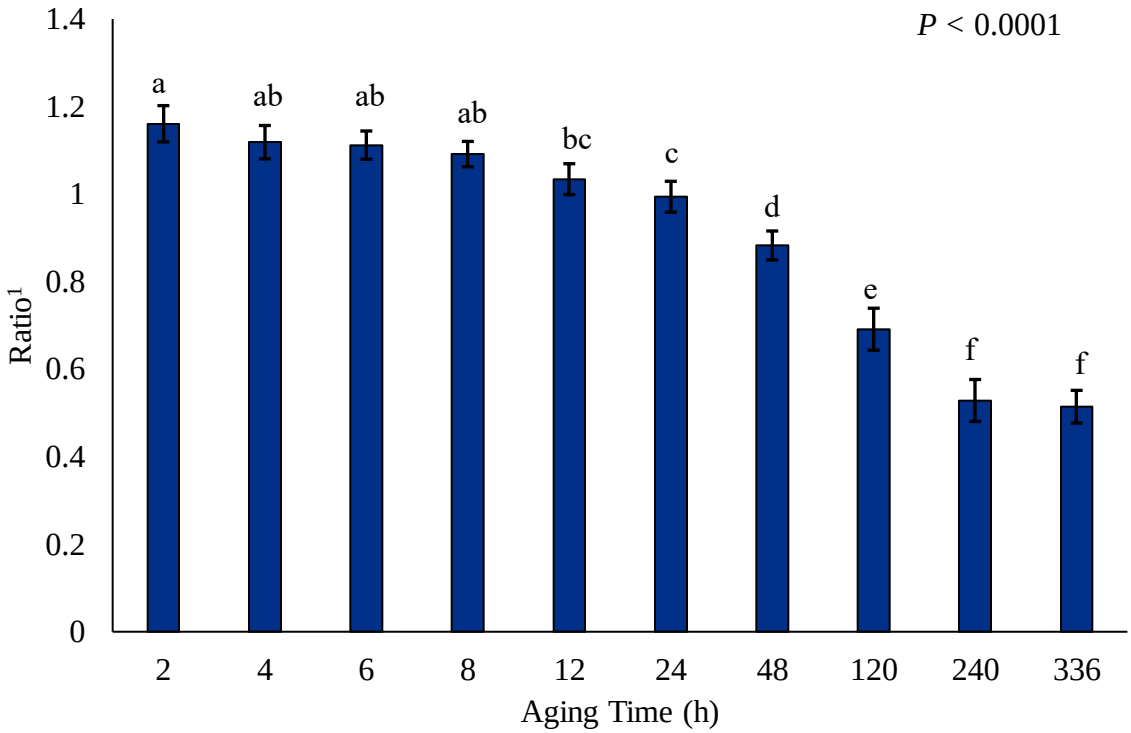
Figure 2.3 - Least square means for the effect of aging time on proteolysis of intact desmin in the *serratus ventralis* (55 kDa)



[1] Expressed as a ratio of intact desmin to an internal standard

[ab] Means lacking common superscripts differ $P < 0.05$

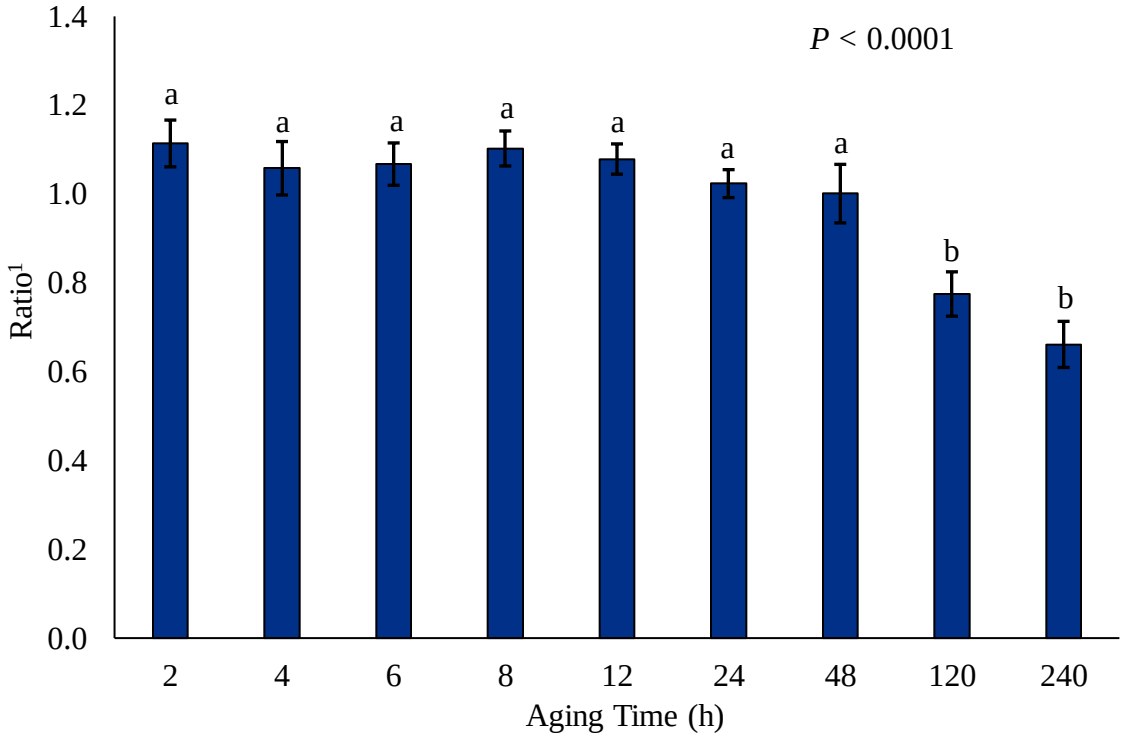
Figure 2.4 - Least square means for the effect of aging time on proteolysis of intact desmin in the *longissimus lumborum* (55 kDa)



[1] Expressed as a ratio of intact desmin to an internal standard

[abcdef] Means lacking common superscripts differ $P < 0.05$

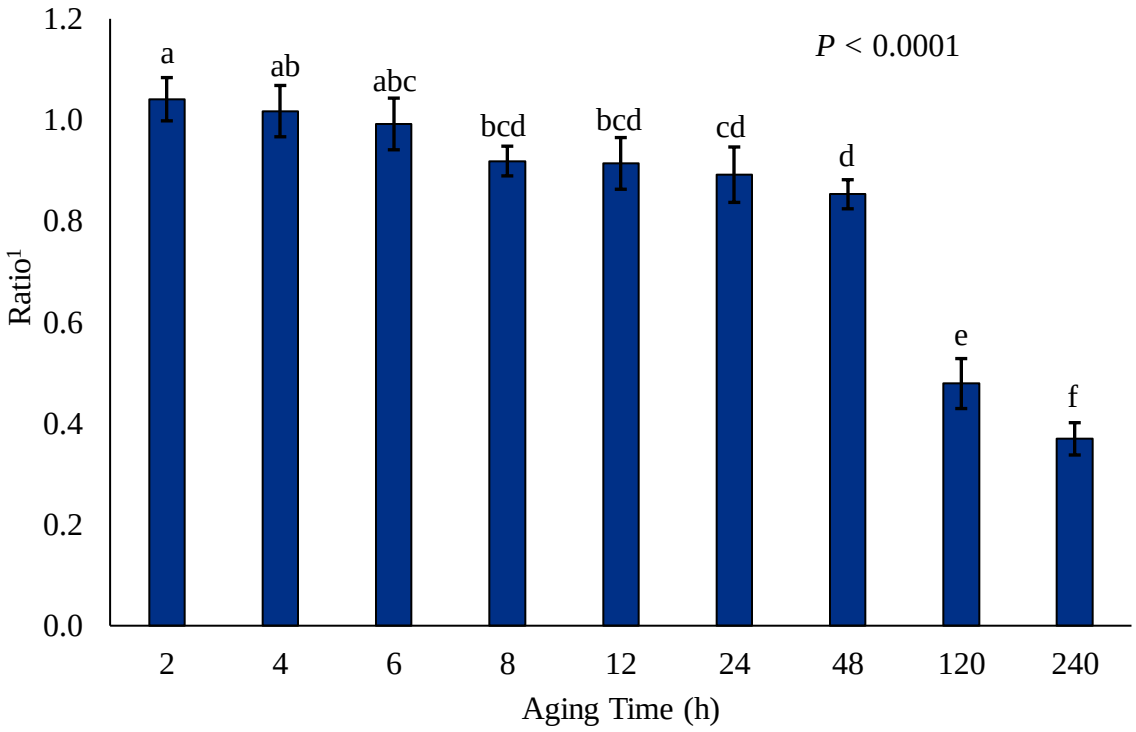
Figure 2.5 - Least square means for the effect of aging time on proteolysis of intact desmin in the *semitendinosus* (55 kDa)



[1] Expressed as a ratio of intact desmin to an internal standard

[ab] Means lacking common superscripts differ $P < 0.05$

Figure 2.6 - Least square means for the effect of aging time on proteolysis of intact desmin in the *longissimus thoracis* (55 kDa)



[1] Expressed as a ratio of intact desmin to an internal standard

[abcdef] Means lacking common superscripts differ $P < 0.05$

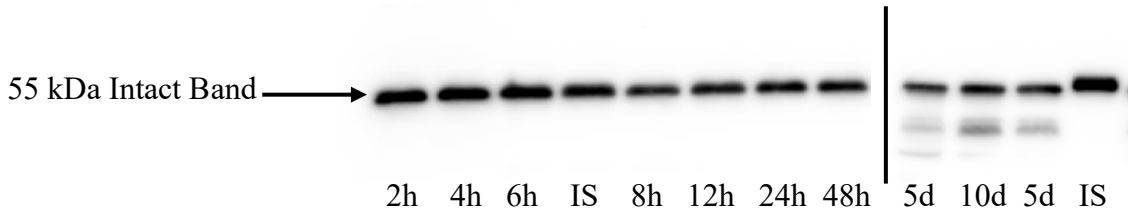


Figure 2.7 - Representative Western blots (2 images, separated by black bar) of intact desmin in aged beef *serratus ventralis* (SV) whole-muscle samples. Intact bands (55 kDa) were compared to corresponding bands of a mixed 2-48 hour aged beef SV internal standard (IS). The same IS was used on all SV blots.

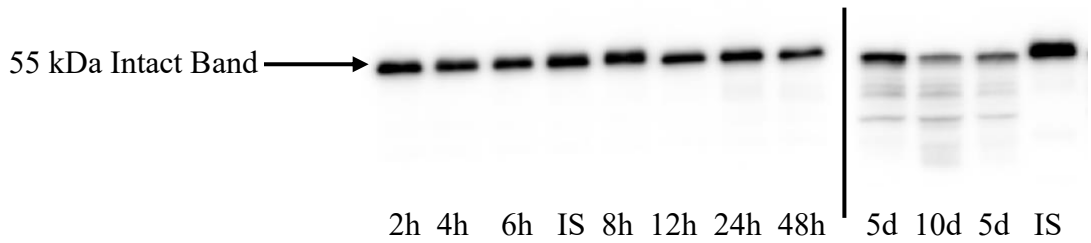


Figure 2.8 - Representative Western blots (2 images, separated by black bar) of intact desmin in aged beef *longissimus thoracis* (LT) whole-muscle samples. Intact bands (55 kDa) were compared to corresponding bands of a mixed 2-48 hour aged beef LT internal standard (IS). The same IS was used for all LT blots.

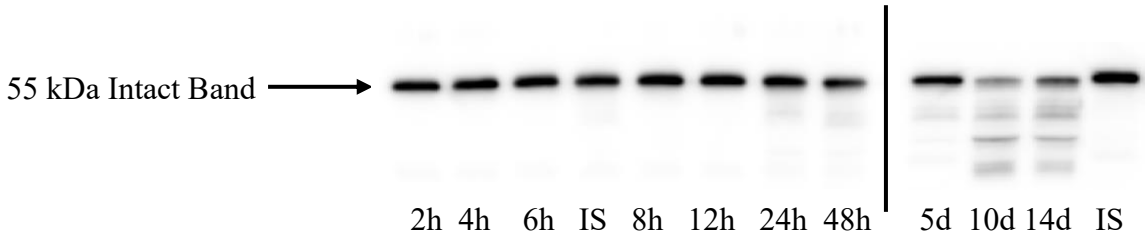


Figure 2.9 - Representative Western blots (2 images, separated by black bar) of intact desmin in aged beef *longissimus lumborum* (LL) whole-muscle samples. Intact bands (55 kDa) were compared to corresponding bands of a mixed 2-48 hour aged beef LL internal standard (IS). The same IS was used on all LL blots.

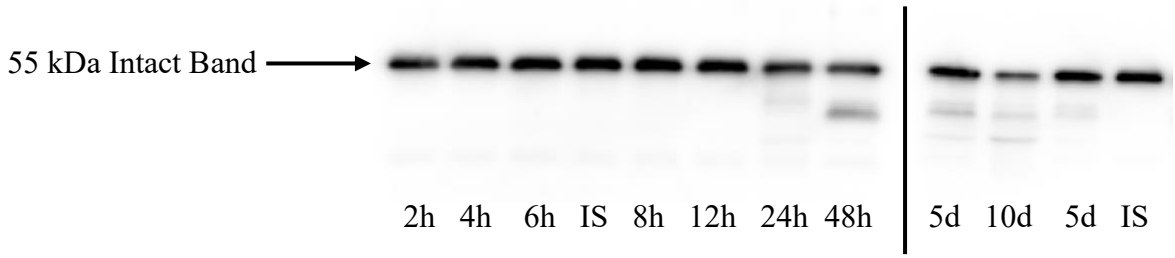
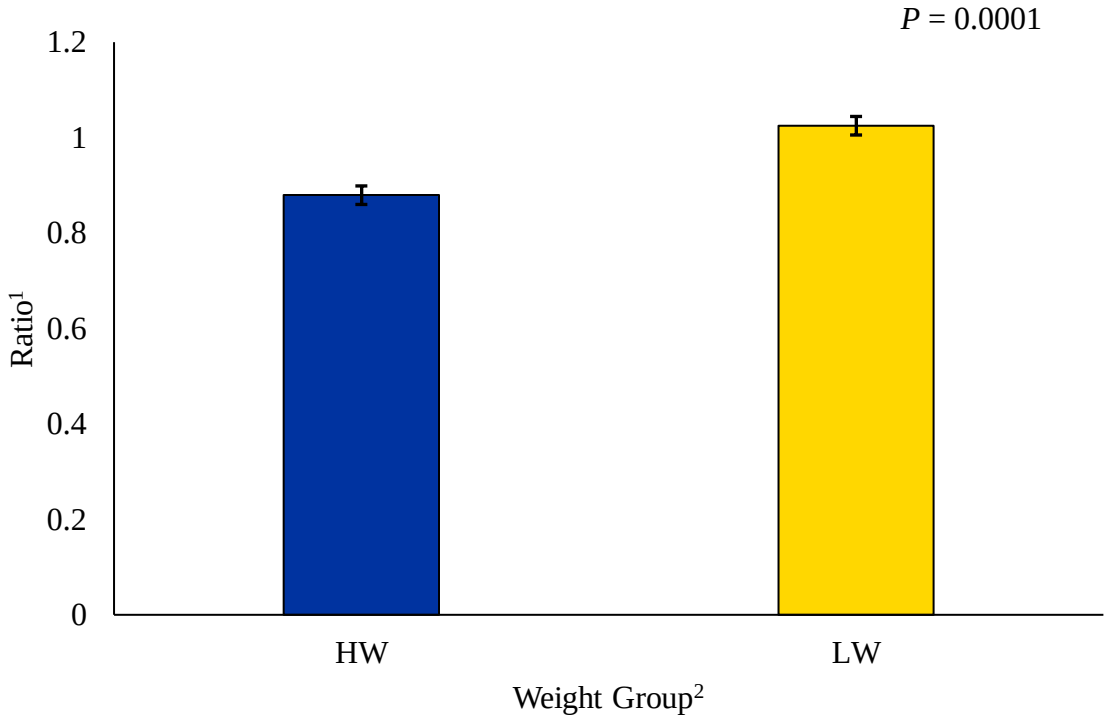


Figure 2.10 - Representative Western blots (2 images, separated by black bar) of intact desmin in aged beef *semitendinosus* (ST) whole-muscle samples. Intact bands (55 kDa) were compared to corresponding bands of a mixed 2-48 hour aged beef ST internal standard (IS). The same IS was used on all ST blots.

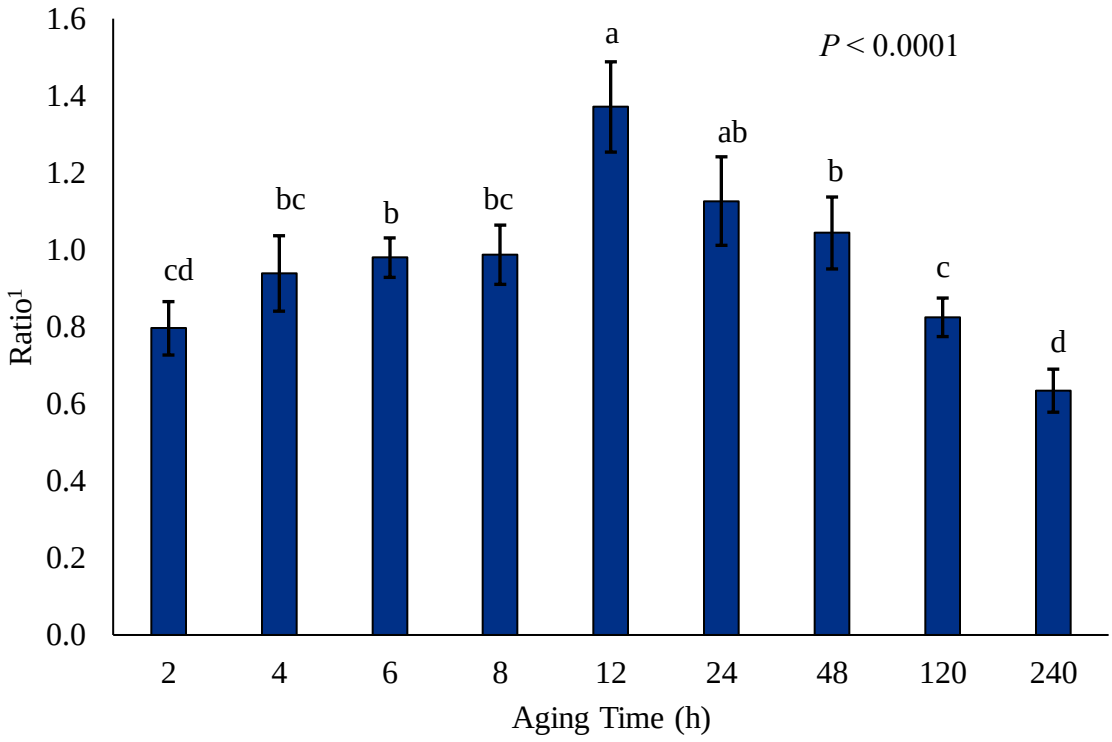
Figure 2.11 - Least square means for the effect of weight group on proteolysis of intact troponin-T in the *semitendinosus* (37 kDa)



[1] Expressed as a ratio of intact troponin-T to an internal standard

[2] heavyweight (HW) group (450 ± 7.6 kg), lightweight (LW) group (349 ± 7.6 kg)

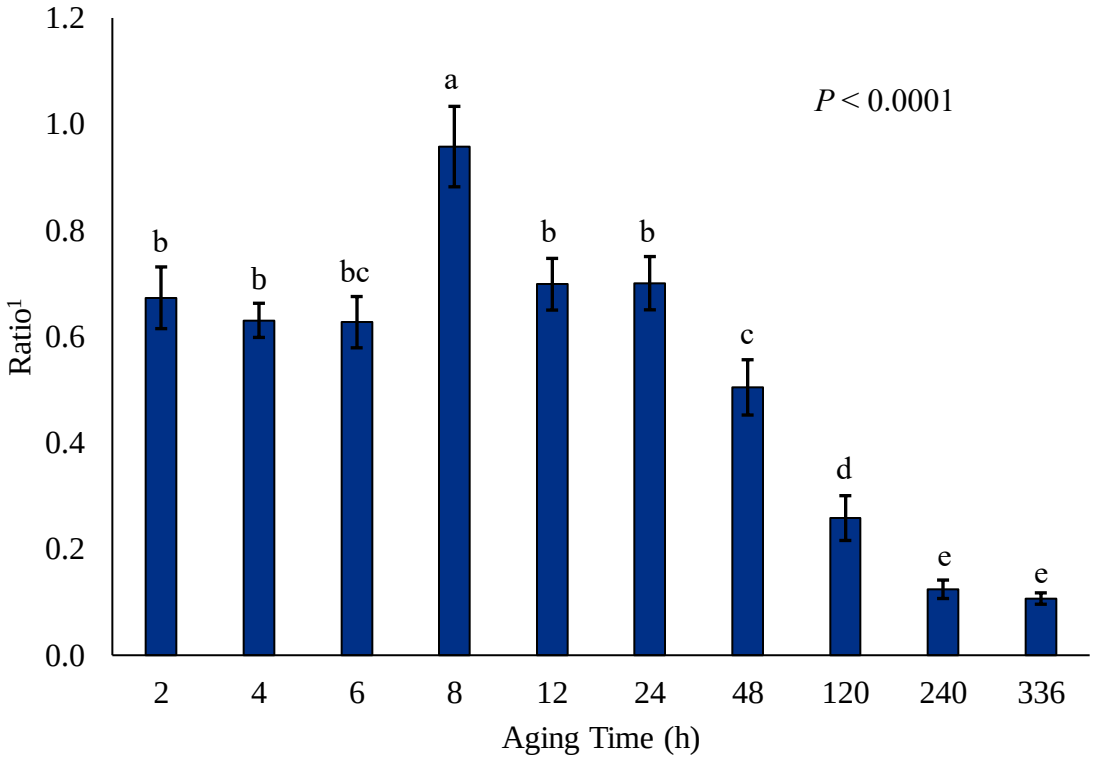
Figure 2.12 - Least square means for the effect of aging time on proteolysis of intact troponin-T in the *serratus ventralis* (37 kDa)



[1] Expressed as a ratio of intact troponin-T to an internal standard

[abcd] Means lacking common superscripts differ $P < 0.05$

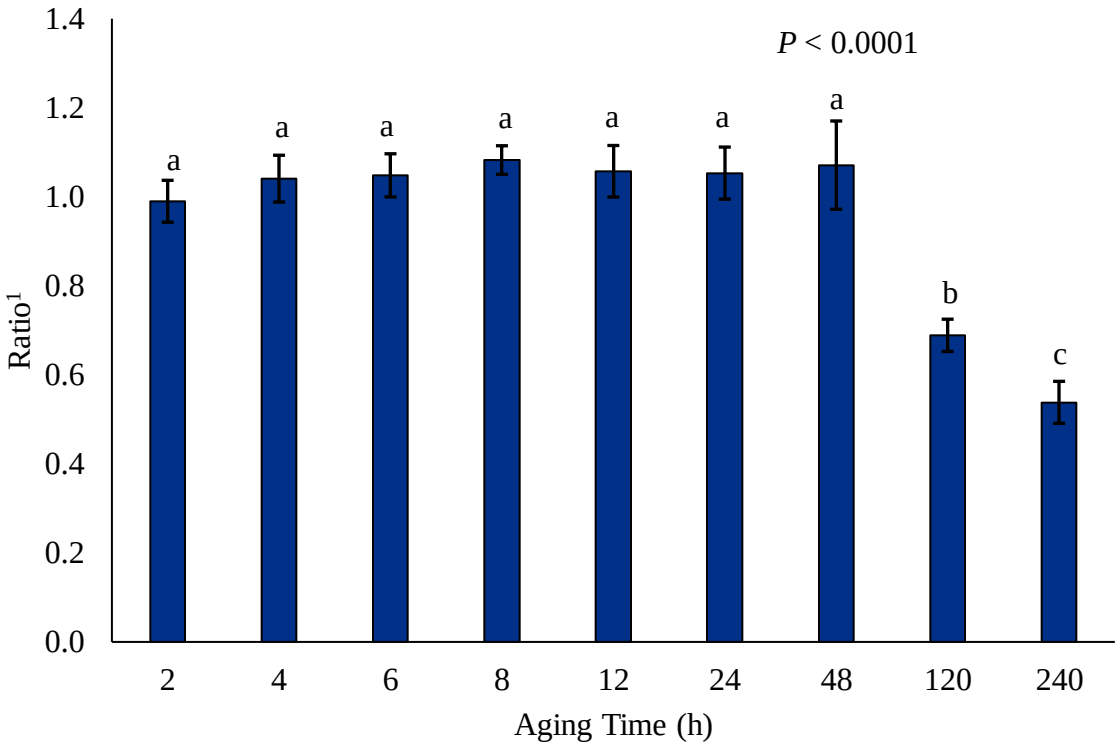
Figure 2.13 - Least square means for the effect of aging time on proteolysis of intact troponin-T in the *longissimus lumborum* (37 kDa)



[1] Expressed as a ratio of intact troponin-T to an internal standard

[abcde] Means lacking common superscripts differ $P < 0.05$

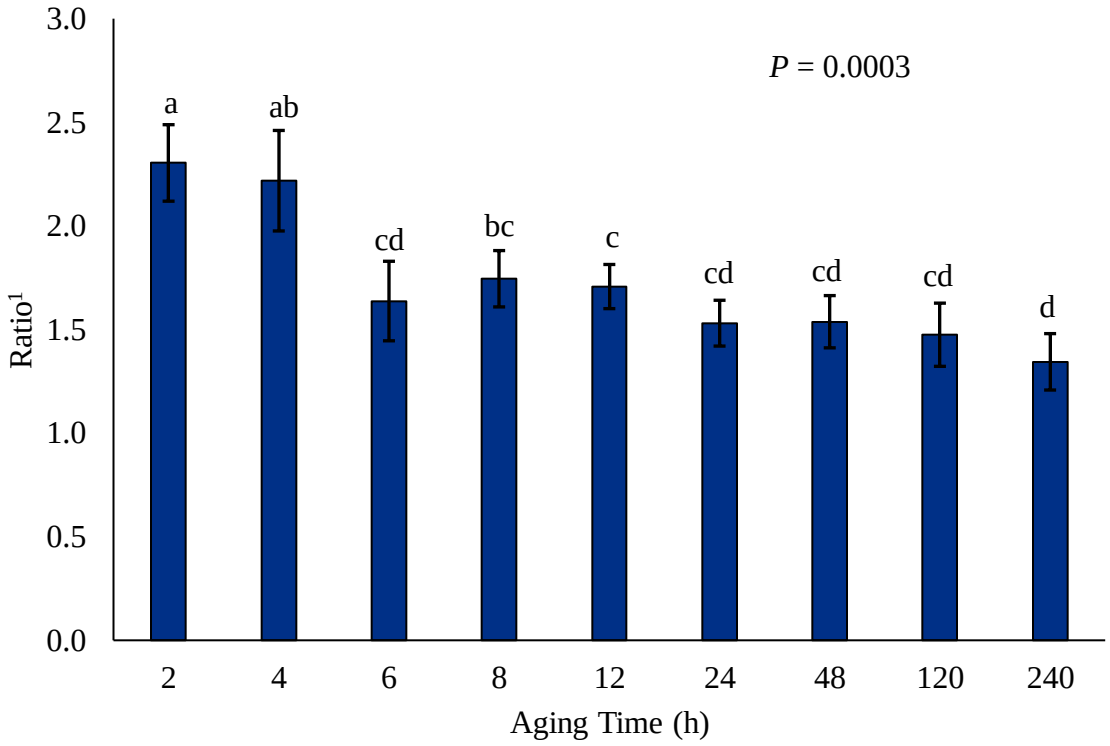
Figure 2.14 - Least square means for the effect of aging time on proteolysis of intact troponin-T in the *semitendinosus* (37 kDa)



[1] Expressed as a ratio of intact troponin-T to an internal standard

[abc] Means lacking common superscripts differ $P < 0.05$

Figure 2.15 - Least square means for the effect of aging time on proteolysis of intact troponin-T in the *longissimus thoracis* (37 kDa)



[1] Expressed as a ratio of intact troponin-T to an internal standard

[abcd] Means lacking common superscripts differ $P < 0.05$

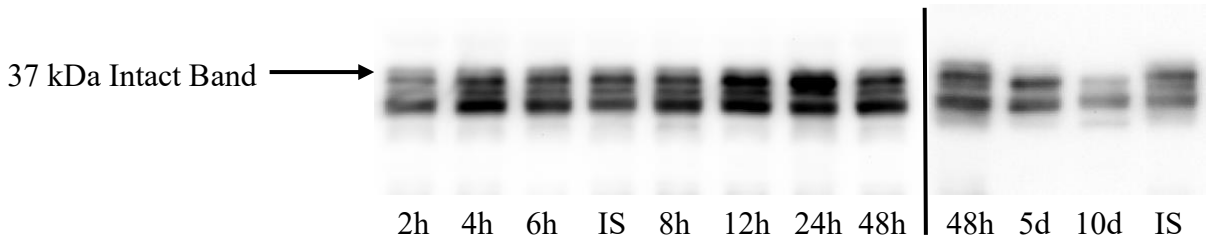


Figure 2.16 - Representative Western blots (2 images, separated by black bar) of intact troponin-T in aged beef *serratus ventralis* (SV) whole-muscle samples. Intact bands (37 kDa) were compared to corresponding bands of a mixed 2-48 hour aged beef SV internal standard (IS). The same IS was used on all SV blots.

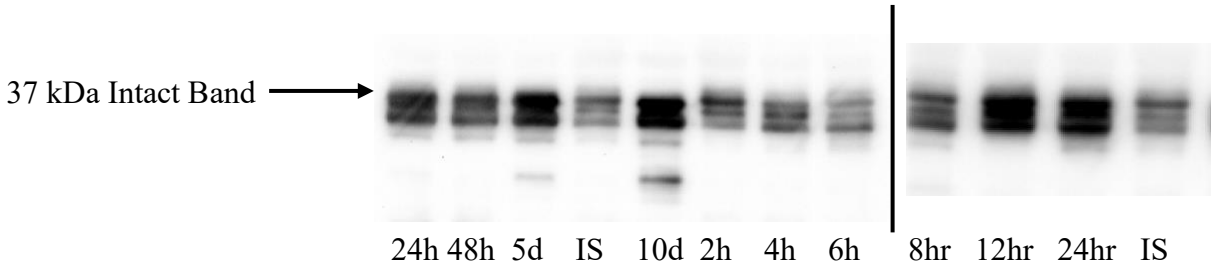


Figure 2.17 - Representative Western blots (2 images, separated by black bar) of intact troponin-T in aged beef *longissimus thoracis* (LT) whole-muscle samples. Intact bands (37 kDa) were compared to corresponding bands of a mixed 2-48 hour aged beef LT internal standard (IS). The same IS was used on all LT blots.

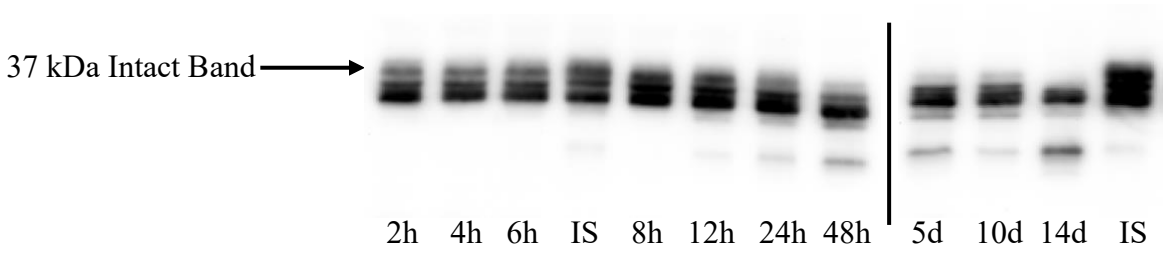


Figure 2.18 - Representative Western blots (2 images, separated by black bar) of intact troponin-T in aged beef *longissimus lumborum* (LL) whole-muscle samples. Intact bands (37 kDa) were compared to corresponding bands of a mixed 2-48 hour aged beef LL internal standard (IS). The same IS was used on all LL blots.

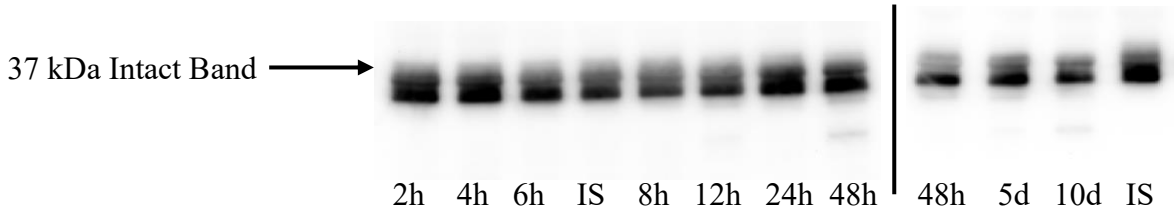


Figure 2.19 - Representative Western blots (2 images, separated by black bar) of intact troponin-T in aged beef *semitendinosus* (ST) whole-muscle samples. Intact bands (37 kDa) were compared to corresponding bands of a mixed 2-48 hour aged beef ST internal standard (IS). The same IS was used on all ST blots.