THE INFLUENCE OF BEEF CARCASS WEIGHT ON TROPONIN-T DEGRADATION AND HEAT SHOCK PROTEIN 70 IN TWO DIFFERENT MUSCLES

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

This study was aimed to assess how carcass size affects protein degradation in postmortem muscle and subsequent impacts on meat quality. Beef carcasses were randomly selected at slaughter and classified as heavy (> 430.9 kg; n = 22), average (351.5 to 385.6 kg; n = 23), or light (< 317.5 kg; n = 20). Troponin-T (TnT) and heat shock protein 70 (HSP70) were analyzed for protein abundance on 3-day aged longissimus (LD) and semimembranosus (SM) muscles and 14-day aged LD muscle. TnT degradation in heavy carcasses was greater in the d-3 SM muscle and less in the d-14 LD muscle. Carcass size was positively correlated with the 30 kDa TnT band in d-3 SM. Therefore, carcass size influenced the TnT degradation but had no influence on HSP70 protein activity in either LD or SM.

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LIST OF ABBREVIATIONS

ADP.....Adenosine diphosphate

ATP.....Adenosine triphosphate

B1D14.....40kDa band TnT at D14

B1D3.....40kDa band TnT at D3

B2D14.....38kDa band TnT at D14

B2D3.....38kDa band TnT at D3

B3D14.....36kDa band TnT at D14

B3D3.....36kDa band TnT at D3

B4D14.....34kDa band TnT at D14

B4D3.....34kDa band TnT at D3

B5D14.....30kDa band TnT at D14

B5D3.....30kDa band TnT at D3

Ca^{2+}.....Calcium ion

CWT.....Carcass weight

ES.....Electrical stimulation

FYG.....USDA final yield grade

HCW.....Hot carcass weight

HSD3.....HSP70 at D3

HSP70.....Heat shock protein 70

HSPs.....Heat shock proteins

LDDL.....Drip loss percentage for LD muscle

LDSF.....Warner Bratzler Shear Force for LD muscle

LM.....Longissimus muscle

MWM.....Molecular weight marker

NaCl .....Sodium chloride

NBQA .....National Beef Quality Audit

NDSU.....North Dakota State University

NFDM .....Non-fat dry milk

PBS .....Phosphate-buffered saline

PH1 .....pH at 45 min

PH2 .....pH at 4 hours

PH3 .....pH at 24 hours

pHu.....Ultimate pH

REA.....Ribeye area

SDS-PAGE .....Sodium dodecyl sulfate polyacrylamide gel electrophoresis

SM.....Semimembranosus muscle

TM1.....Temperature at 45 min

TM2.....Temperature at 4 hours

TM3.....Temperature at 24 hours

TnT.....Troponin-T

TwelvRF .....Twelve rib fat thickness

US .....United States

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Introduction

Currently, carcass size is one of the most important issues in the meat sectors, especially in beef production. In the United States (U.S.), beef production has increased slightly since 1977 to 2015 (Maples et al., 2018). This increased production has occurred with 13 million fewer cattle (Boykin et al., 2017). In the USA, carcass weight increases about 1 to 1.5 kg per year (Savell et al., 2012). Since National Beef Quality Audit (NBQA) -1995, there has been a continued increase in hot carcass weight (Boykin et al., 2017). Between 1991 to 2011 audits, the average increased carcass weight 761 Ibs. to 825 Ibs. In 2011, National Beef Quality Audit listed weight and size as one of the top six quality challenges (Igo et al., 2013). Therefore, carcass size and weight remain an important issue in the meat sector.

Increased carcass weight can be accomplished by several factors including carcass size, improved nutrition supplementation, better genetics, growth promotion technologies, and economic condition (Lusk, 2013). Bunting (2015) discussed potential reasons for carcasses continuing to get heavier, with processing facilities' labor costs and cattle availability at the forefront. In the case of Zilmax-fed cattle, weights increase about 10 and 15 kg for live and carcass weight, respectively (Delmore et al., 2010). A positive estimation of genetic correlation was found between direct genetic effects for weaning weight and hot carcass weight (Crews and Kemp, 1999). Findings from few literatures approved that, improvement of carcass size strongly dependent on these selected factors.

National Beef Quality Audit (NBQA) was first conducted in 1991, and it has been repeated every 5 years to see the changes and what areas need to be focused on the beef industry. From the beginning, NBQA maintained roles for analyzing and documenting quality and

consistency of the U.S. fed steer and heifer beef industry (Boykin et al., 2017). They also reported that continued increases in hot carcass weight (HCW) were affecting steak thickness due to portioning. Specifically, from 1991 to 2011, as HCW increased, average ribeye area increased from 12.9 square inches (in^2) to 13.8 in^2. However, consumers prefer thicker steaks with a smaller surface area (Maples et al., 2018)

Literature Review

Meat quality

Hoffman (1987) stated that meat quality is "the sum of all quality factors of meat in terms of the sensoric, nutritive, hygienic, toxicological and technological properties". Sensory properties include tenderness, color, flavor, odor, and juiciness while nutritive factors include fat and protein content as well as vitamins, minerals and biological value. Hygienic and toxicological factors include spores, molds, bacteria, toxins and residues, and finally technological factors include water-holding capacity, pH, water distribution, etc (Troy, 1999). Of all of the quality factors, research has shown that sensory attributes – tenderness, flavor, and juiciness influence consumers overall perceptions of the taste of beef (Neely et al., 1998). In fact, these sensory attributes are the most important value-determining factors affecting consumers meat purchasing decisions (Savell et al., 1987; Smith et al., 2008).

Tenderness

Tenderness is an important meat quality trait and the biological, structural, and physiological mechanisms underlying meat tenderness have been extensively investigated and reviewed (Dransfield et al., 1981; Koohmaraie, 1988; Tornberg, 1996). Meat tenderness is determined by the amount and solubility of connective tissue, sarcomere shortening during rigor development, and postmortem proteolysis of myofibrillar and myofibrillar-associated proteins

(Goll, 1991). Proteolysis is the major determinant of longissimus tenderness, sarcomere length is the major determinant of psoas major tenderness, and connective tissue is the major determinant of biceps femoris and semimembranosus muscles tenderness (Koohmaraie et al., 2002). Meat tenderness is associated with ultimate pH, temperature, and protein degradation (Huff-Lonergan et al., 1996a)

Muscle temperature during aging is an important contributor for development of meat tenderness (Lee, 1986). Aging muscle at high temperatures caused decreased pH and decreased protein solubilities (Bruce and Ball, 1990). High temperature aging (30 to 40°C) caused heat shortening could affect tenderness through the shortening of sarcomeres and decreased protein solubility (Busch et al., 1967; Lee and Ashmore, 1985; Koh et al., 1987). Bruce and Ball (1990) concluded that during early postmortem, a moderate temperature produced a slow decline in pH, which should prevent heat contraction. Muscle temperature has been an important contributor for development of meat tenderness during aging (Lee, 1986).

There is a general positive correlation between sensory tenderness and juiciness (Shackelford et al., 1995; Otremba et al., 2000; Silva et al., 2007; Guzek et al., 2012). Juiciness is highly dependent on the moisture retention ability of cooked meat, but interestingly, the lack of a strong correlation was found between perceived juiciness and water-holding capacity of meat, an indicator of meat tenderness (Hughes et al., 2014). Most recently, an improvement of juiciness coincided with the early activation of calpain-2, which suggests postmortem proteolysis may play a role in juiciness development (Colle et al., 2018). Further studies are required to see the inter-relationship between the muscle protein thermal denaturation behavior, functionality, and juiciness of aged meat (Kim et al., 2018).

Impact of carcass size on meat quality

The most recent National Beef Quality Audit listed weight and size as one of the top six quality challenges (Boykin et al., 2017). Larger size carcasses mean larger product size for muscle cuts such as steaks and results in more pounds of meat per animal. Industry has been hearing from consumers for at least two decades that they are not interested in larger size steaks. In addition, recently published research from Oklahoma State University confirms that consumers want thicker steaks and thinner cut steaks have a negative impact on steak demand (Peel, 2017). Although the industry has improved carcass quality, increased carcass size was a concern (Boykin et al., 2017). In 2017, beef supply reduced due to a drop-off in carcass weights (Peel, 2017). However, a recent South African feedlot system determined that heavier carcasses improved meat quality in terms of shear force but had increased drip loss, which marginally reduced carcass yield (Agbeniga and Webb, 2018). Although some beef products (e.g., ground beef) are largely unaffected by changing carcass size, weight and cross sectional area of cuts from subprimals, such as the longissimus muscle (LM), the muscle containing the ribeye, are highly correlated with carcass size (Peel, 2017). Industries are seeking to identify clearly which size of carcasses favors best for meat quality attributes and consumers acceptability.

According to Ferguson and Gerrard (2014), glycolytic rate and energy metabolism play a crucial role in transforming living muscle into high quality meat. Glycolytic rate is a function of the rate of pH decline, cooling rate (which is affected by subcutaneous fat), residual muscle glycogen content, and plasma insulin level at slaughter (Warner et al., 2014). According to Thompson (2002) heavier carcasses passed through the heat shortening window ($pH > 6$ when temperature is above 35°C). Warner et al. (2014) indicated that heavier carcasses passed through the heat shortening window due to insulation from higher subcutaneous fat thickness and faster

glycolytic rate. Agbeniga and Webb (2018) reported that at 45 min, 3, 6, 12 and 24 h postmortem, heavier carcasses exhibited faster pH decline and slower temperature decline at all-time measurements. In a study with electrical stimulation (ES) on carcasses, Savell et al. (1979) showed that ES (400 V) produced higher ratings for tenderness, flavor, color and overall palatability compared to non-ES sides on heavy carcasses. Electrical stimulation depletes energy reserves in muscle at a faster rate through glycolysis and reduces the risk of sarcomere shortening (Agbeniga and Webb, 2018). However, to achieve similar glycolytic potential, heavier carcasses require less ES than lighter carcasses (Thompson, 2002).

Conversion of muscle to meat

The muscle to meat conversion is a gradual degradative process where several biochemical and physical changes take place (Matarneh et al., 2017). This process begins in the muscle immediately after harvest when muscle glycogen is metabolized by anaerobic glycolysis and phosphorylates ADP to ATP. Anaerobic glycolysis produces a waste product called lactic acid (Maltin et al., 2003). Normally, in the case of live animals, lactic acid is transferred from blood to the liver. During conversion of muscle to meat, lactic acid increases in the muscle due to the absence of blood (Shen and Du, 2015). So, this lactic acid causes lowering of the intracellular pH in the muscle, and within 24 h post-mortem, the pH becomes an ultimate pH (pHu) in the normal range of 5·4-5·7 for enzymatic activity. Within muscle, the buildup of lactic acid releases calcium and causes contraction into the muscle. Furthermore, muscle is sensitive to ATP, which is needed for muscle relaxation. This contraction and relaxation process continues until all the energy of the muscle is used. As a result of these processes, the reduced energy levels and increased calcium levels, rigor mortis occurs in the tissue (Maltin et al., 2003)

Proteolysis and aging

Proteolysis is a process involved in muscle to meat conversion, and changes in meat tenderness are due to changes in the properties of muscle fiber and connective tissue (Maltin et al., 2003). During aging of meat, tenderization mechanisms are primarily known as enzymatic in nature and involve intracellular proteolytic systems capable of postmortem proteolysis (Ouali et al., 2006). Proteolysis is the breakdown of proteins into smaller elements contained polypeptides or amino acids. The proteolytic process is accomplished by enzymes known as proteases, which is responsible for meat aging and function in living muscle (Toldrá, 2017).

For postmortem proteolysis, a specific protease is considered where three specific criteria must be included (Goll et al., 1983; Koohmaraie, 1994). These criteria for any participating protease are: must be localized within the skeletal muscle cell, have access to the myofibrillar and/or costameric proteins, and can degrade the same proteins that are degraded during postmortem proteolysis. Based on those criteria, several endogenous proteolytic systems contribute to postmortem proteolysis including calpain system, the lysosomal proteases, and cathepsins. These systems have the capability of degrading myofibrillar and cytoskeletal proteins (Dransfield, 1994). However, researchers reported that the lysosomal system plays little to no role in postmortem proteolysis.

During aging, proteolysis of cytoskeletal protein (titin and nebulin) and intermediate filaments (desmin) occurs due to the calpain system (Huff-Lonergan et al., 1996a). Studies suggested that the calpain system is recognized as the major driver of postmortem proteolysis (Koohmaraie et al., 1991). In the family of cysteine proteases, the main enzymes include calpain 1 and 2 and their endogenous inhibitor calpastatin. Calpain 1 and calpain 2 are composed of heterodimers which have two subunits with molecular weight of 80 kDa and 28 kDa. The large

subunit of calpain 1 and calpain 2 share 50-60% sequence homology and 28 kDa small subunits are identical both calpain 1 and calpain 2 (Ohno et al., 1990; Carafoli and Molinari, 1998). They are calcium activated proteases required micromolar Ca^{2+} and millimolar Ca^{2+} concentration respectively for their proteolytic activation (Kim et al., 2018). During aging period, they hydrolyze myofibrillar and cytoskeletal proteins including titin, nebulin, filamin, desmin and troponin-T which is related to the meat tenderization process (Huff-Lonergan et al., 1996a). The major difference of them mainly based on calcium requirement. Calpain 1 needs much less Ca^{2+} (half-maximal activity, 3 to 50 µM) to be activated than calpain 2 (half-maximal activity, 400 to 800 µM (Upla et al., 2008). Colle and Doumit (2017) reported that calpain 1 and calpain 2 both have contribution for beef tenderness while calpain 1 is responsible for improving tenderness during early postmortem and calpain 2 is responsible for improvement after 14 days of aging due to activation of calpain 1 at first 14 days of aging and calpain 2 activation occurs in Longissimus lumbroum and SM muscles by 14 days of aging and for all muscles by 28 days. The rate of activation and autolysis of calpain-1 could alter the quantity of protein degradation that occurs during aging (Carlson et al., 2017). Autolysis is the process of destruction of cells or tissues by their own enzymes. In the presence of calcium, calpain 1 and calpain 2 can autolyze (Suzuki et al., 1981a). One of the significant roles of calpain autolysis in postmortem muscle is that, it can decrease calcium concentration required for their proteolytic activation (Suzuki et al., 1981b). Some of the studies indicated that, autolysis of calpain 1 and calpain 2 begins with their proteolytic activities (DeMartino et al., 1986; Saido et al., 1994). However, complete autolysis of calpain1 and 2 can loss their proteolytic activity by destroying integral structure (Elce et al., 1997).

During early postmortem, rate of pH decline is an important factor for calpain 1 activation and autolysis (Melody et al., 2004; Carlin et al., 2006). . Lower muscle pH is due to the accumulation of lactic acid. Accumulation of muscle lactic acid in the early postmortem period can adversely affect meat quality. Development of a low pH (acidic) in muscle, before natural body heat and heat of continuing metabolism have been dissipated through carcass chilling, causes denaturation of muscle protein. Thomas Vilgis stated that, “ongoing protein denaturation over a long period of time might lead to undesired properties such as a “lack of tenderness or juiciness”. Muscles that maintain a high pH during conversion of muscle to meat may be very dark in color, and very dry on the exposed cut surface because naturally occurring water is tightly bound to proteins.

A rapid decline pH reduce calpain autolysis and proteolysis of their substrates (Bhat et al., 2018). However, slightly decline pH in the muscle may lead to improve tenderness and also have an advantage in water holding capacity (Melody et al., 2004; Simmons et al., 2008).In addition, studies reported that, rapid pH decline completely arrested calpain 1 autolysis and degradation of desmin and talin (Bee et al., 2007; Barbut et al., 2008)., Melody et al. (2004) explained that the rate of postmortem pH declines within the first 6 h after exsanguination influences the rate of calpain 1 activity and autolysis and may play a pivotal role in regulating early postmortem proteolysis. Rhee et al. (2006) imposed three different temperature conditioning methods and detected no differences in muscle temperature or calpain 2 activity. However, calpain 1 is very sensitive to temperature (Du et al., 2017). Activation of calpain 1 was faster in lamb at 25-35°C temperature (Geesink et al., 2000)and at 36°C temperature in beef (Hwang et al., 2004) by using immunoblot analysis of muscle incubated for 24 h. Although in beef muscle faster proteolysis was observed when enter rigor at 15°C up to 3 days post-mortem

(Thomson et al., 2008). Hwang et al. (2003) reported that electrical stimulation accelerates proteolysis through Ca^{2+} activation that activates calpain enzyme activity. Furthermore, they provided an explanation for some intrinsic effect, which was associated with rapid pH decline at high temperature that affected the calpain/ calpastatin ratio. However, Dransfield et al. (1992) concluded that temperature did not affect calpain activity until the pH reached 6.2.

Current evidence suggests that proteolysis of key myofibrillar proteins is responsible for meat tenderization. These proteins are involved in: (1) inter-myofibril linkages (e.g., desmin and vinculin), (2) intra-myofibril linkages (e.g., titin, nebulin, and possibly troponin-T [TnT]), (3) linking myofibrils to sarcolemma by costameres (e.g. vinculin and dystrophin), and (4) the attachment of muscle cells to the basal lamina (e.g. laminin and fibronectin). The function of these proteins is to maintain the structural integrity of myofibrils (Price, 1991). Proteolytic degradation of these proteins would cause weakening of myofibrils and thus tenderization (Koohmaraie et al., 2002).

Proteolysis of desmin and TnT

The TnT subunit is the component of tropomyosin-binding that constitutes the elongated portion of the troponin complex (Bhat et al., 2018). TnT is a key regulatory protein and indicator of overall postmortem proteolysis and is involved with the calcium-dependent regulation of skeletal muscle contraction. Degradation of troponin T is closely associated with meat tenderization (Macbride and Parrish, 1977). Specifically, accumulation of the 30 kDa fragment of TnT is correlated to increases meat tenderization during aging (Harris et al., 2001). Penny and Dransfield (1979) reported that when degradation of TnT improves during postmortem aging, meat tenderization concurrently improves. Degradation of this protein could demonstrate fragmentation of the myofibrils by disrupting its interaction with other actin filament

components (Bhat et al., 2018). Studies over the last 20 years have used in vitro models to demonstrate the degradation of TnT is directly linked to calpain activity (Huff-Lonergan et al., 1996a; Baron et al., 2004; Lametsch et al., 2004). Thus, the loss of troponin T is an indicator of postmortem proteolysis of the key myofibrillar proteins (Contreras-Castillo et al., 2016). Iwanowska et al. (2010) reported that TnT can be applied in proteomics as a marker for the proteolysis of beef. Contreras-Castillo et al. (2016) reported that the faster degradation of TnT in the high ultimate pH (pH_u) group may be related to higher calpain 1 activity which is close to the optimal pH of the enzyme. Furthermore, degradation of intact TnT by calpain 1 provides polypeptide segments of 28 and 30 kDa (Bhat et al., 2018). The extent of these concurrent polypeptides strongly correlates with the meat tenderness during postmortem aging (Rowe et al., 2003).

Cytoskeletal protein desmin is a distinct organizational intermediate filament (Bhat et al., 2018) that is found in the costamere, transmembrane plaques and in association with the Z line (Koohmaraie et al., 1991). In mature muscle fibers, the network of intermediate filaments are mainly composed of the protein desmin (Lazarides and Hubbard, 1976). Desmin acts as a substrate for the action of both calpain 1 and calpain 2 (Taylor et al., 1995) and maintains structural and functional integrity by providing mechanical strength to the muscle (Bhat et al., 2018). They also reported that since the breakdown of desmin protein plays a role in the early establishment of sarcomere structure it has been considered as one of the earliest markers of mammalian skeletal muscle. In addition, desmin plays an important role in the improvement of tenderness, water holding capacity, and juiciness of meat through cleavage in the costameres by calpains during early postmortem (Kristensen and Purslow, 2001). Like TnT, desmin degradation has been directly linked to calpain activity using in vitro models of postmortem muscles (Huff-

Lonergan et al., 1996a; Baron et al., 2004; Lametsch et al., 2004). Evidence suggests that desmin is almost completely degraded at 7 days post-mortem aging in beef muscle (Muroya et al., 2010; Kemp and Parr, 2012), which has a significant influence on meat tenderization by affecting the shear force and water holding capacity of meat (Taylor et al., 1995; Kristensen and Purslow, 2001; Huff Lonergan et al., 2010).

Heat shock proteins (HSPs) and meat tenderness

According to the molecular size, HSPs are classified into five categories including HSP60, HSP70, HSP90, and HSP100, respectively (Fink, 1999). A fifth class of the HSP family is small heat shock proteins (sHSPs). Small heat shock protein is smallest of the heat shock protein family (Lomiwes et al., 2014). The sHSPs are variable in size ranging from 12 to 43 kDa (Haslbeck et al., 2005). The family of HSP is well known to all due to its role in cell protection (Carvalho et al., 2014). An excess amount of HSPs plays an anti-apoptotic role in the muscle that could inhibit meat aging and consequently cause a negative effect on meat tenderness (Ouali et al., 2006). HSPs prevent the apoptotic process in multiple ways. According to Beere (2005) and Lomiwes et al. (2014), HSP70 and HSP90 interfere with the interaction of Apaf-1 and procaspase 9, thus impeding the continuation of apoptosis. An example of sHSPs is HSP27 is known as heat shock beta 1, which is involved in cellular organization (Creagh et al., 2000). Further HSP27 interacts with myofilaments (Fischer et al., 2002; Blunt et al., 2007). Besides that, HSP27 has been shown to delay initiation of proteolysis during 7- and 14-days post slaughter as during the progression of the meat aging process it can contribute to meat tenderness (Morzel et al., 2008).

HSP70 is as one of the differently expressed proteins (Carvalho et al., 2014). Creagh et al. (2000) reported that HSP70 has also shown anti-apoptotic function. According to Carvalho et

al. (2014) Presence of less HSP70 in animals indicates lower meat shear force value. To maintain meat quality, HSP27 and HSP70 play a crucial role in the heat shock protein family through the consideration of meat tenderness during the maturation process (Ouali et al., 2006).

There is a weak correlation between fat thickness and muscle pH. For carcasses with less than 0.76 cm of back fat, meat is darker and muscle pH higher than carcasses with more than 0.76 cm of back fat (Tatum et al., 1982). In addition, research has found that leaner carcasses produced less tender beef steaks, and t there seemed to be a threshold at approximately 0.76 cm of back fat below which carcasses produced less tender beef. The findings of Tatum et al. (1982) and Page et al. (2001) show the relationships between muscle pH/color and beef tenderness. This relationship indicates that beef carcasses with less than 0.76 cm of back fat have less tender beef than fatter carcasses due, at least in part, to differences in postmortem metabolism.

The value of carcasses is mainly dependent on carcass composition, including fat, bone, and proportion of muscle (Pesonen et al., 2012). Oprządek et al. (2001) showed that a superior carcasses must have a high proportion of muscle with a low proportion of bone and an optimum level of fat. Agbeniga and Webb (2018) reported that different carcass size has a positive and negative impact on meat quality due to variation of the postmortem processes. A relationship between subcutaneous fat thickness and glycolytic rate in muscles has been reported in some studies (Hopkins et al., 2007; Warner et al., 2014). Thicker muscles may have a higher risk of high rigor temperature and entering the heat shortening window due to insulation from the higher subcutaneous fat thickness and faster glycolytic rate (Warner et al., 2014). This condition induces the exhaustion of proteolytic enzyme activity at a high temperature and low muscle pH, which could reduce meat tenderness (Simmons et al., 1996). However, there is a lack of

information on proper handling procedures for large size carcasses, which may impact meat quality parameters.

CHAPTER 2. THE INFLUENCE OF BEEF CARCASS WEIGHT ON TROPONIN-T DEGRADATION AND HEAT SHOCK PROTEIN 70 IN TWO DIFFERENT MUSCLES

Abstract

This study was aimed to evaluate carcass size effects on protein degradation and subsequent impacts on meat quality. Heavy (> 430.9 kg), average (351.5 to 385.6 kg), and light (< 317.5 kg) weight beef carcasses (n = 65) were randomly selected over 5 different collection days at a commercial abattoir. Carcasses were monitored for temperature and pH changes at 45 minutes, 4 hours, and 24 hours. At those time points, internal temperature, and pH of the *semimembranosus* (SM) and *longissimus* (LD) between the 12th and 13th rib was measured in five carcasses per weight class per day using a hand-held thermometer and pH probe. After an approximate 24 h chill, carcass data including ribeye area, 12th rib fat, and quality and yield grades were evaluated by trained personnel. Upon fabrication, samples were transported to the North Dakota State University meat laboratory where a 2.5 cm steak was collected from the cranial end of the ribeye roll and the proximal end of inside round and aged for 14 d for shear force. Muscle samples from the LD and SM were collected at 3 days and 14-days of aging. Western blots for troponin-T (TnT) and heat shock protein 70 (HSP70) were performed on 3-day and 14-day aged LD muscle, and 3-d aged SM muscle samples. Reactive bands for TnT were observed at 40 kDa, 38kDa, 36 kDa, 34 kDa, and 31 kDa. LSMeans were measured using proc mixed procedure of SAS and tukey test was performed for mean comparison. Pearson correlation coefficients were determined using PROC CORR of SAS. Carcass size did not influence (P > 0.05) TnT degradation and HSP70 protein abundance in the LD and HSP70 protein abundance in the SM. However, in the d-3 SM, heavy carcasses had less abundance (P = 0.02) of a 40 kDa TnT band compared to light carcasses. Also, in the d-3 SM, heavy carcasses had greater

abundance of a 34 kDa (P = 0.03) and a 30 kDa (P = 0.03) TnT band than light carcasses. However, in the d-14 LD, light carcasses had greater abundance of a 30kDa (P = 0.007) band compared to heavy carcasses. Carcass size was negatively correlated with the 30 kDa TnT band in d-14 LD (r = -0.348, P= 0.01). Expectedly, carcass size was positively correlated with the 30 kDa TnT band (r= 0.430, P = 0.04) and negatively correlated with the 40 kDa TnT band (r = -0.436, P = 0.03) in d-3 SM. The 4-hr pH was negatively correlated with 30 kDa TnT band (r = -0.425, P = 0.04) in the d-3 SM. In addition, ribeye area was negatively correlated with the 30 kDa TnT band at d-3 (r = -0.321, P = 0.02). Moreover, drip loss percentage and shear force were also negatively correlated with the 30 kDa TnT band in d-14 LD (r = -0.270, P = 0.04; r = -0.307, P = 0.03, respectively). Troponin-T degradation was influenced by carcass size in the SM but not the LD. HSP70 protein was not influenced by carcass size at both muscle types (LD and SM). However, in the LD, correlations between the 30 kDa TnT band and carcass size as well as ribeye area were negative indicating that the LD would have less protein degradation as the carcasses got larger. In the SM, there was an opposite observation where the larger carcasses had more accumulation of the 30 kDa TnT degradation product. This does not seem to be temperature or pH dependent as calculated correlations between temperature, pH, and TnT bands trended similarly in both the LD and SM. However, it seems that the LD and SM respond differently to increases in carcass size and impacts on postmortem protein degradation.

Introduction

According to USDA ERS data in 2019, cattle production is one of the most significant agricultural industries in the United States, which made up $66.2 billion cash receipts in 2019. United States is the largest beef consuming country in the world in 2018 followed by China and Brazil (Source: USDA FAS data, 2018). The primary driver of beef consumption is that

consumers enjoy the taste and eating satisfaction of beef. Consumers usually evaluate beef palatability based on their perception of tenderness and juiciness. Therefore, ensuring a supply of high-quality meat is essential to maintain beef's market share, which benefits farmers and ranchers. Measurable and observable characteristics of the beef carcass are used to determine the value of beef. Proteolytic degradation of structural proteins during aging plays a significant role in the development of meat tenderness. Variability in carcass size and weight is posing challenges for beef producers for a variety of reasons, including inconsistencies in product quality and eating satisfaction (Boykin et al., 2017). Previous studies indicate that carcass size has significantly increased over the years due to continual improvements in genetic selection, technologies, nutrition, health, and welfare, low cattle numbers, and demand for more beef (Capper, 2011; Dahlen et al., 2014; White et al., 2015; Sneeringer et al., 2017). According to USDA livestock slaughter report in 2014, the average weight of cattle slaughtered in the United States increased from 2011 to 2014. There was a sharp rise of average dressed weight of U.S. slaughtered cattle from 799 lbs/head to 822 lbs/head between September 2013 and September 2014. The market has changed to reflect these increased slaughter weights by lowering discounts for heavier carcasses. Along with these increased carcass weights, subcutaneous fat, and to a lesser extent, ribeye areas have increased as well (Igo et al., 2013). Bunting (2015) explains that increased carcass size and decreased carcass numbers can improve sustainability by producing more beef with the same number of resources. The dynamic between chilling and postmortem metabolism and effects on the eating quality of meat from these larger carcasses has not been highly investigated, and much of the information found in the literature is dated and not necessarily applicable to the larger carcasses produced today (Saleem and Majeed, 2014). Furthermore, there are contradictory reports where Shorthose and Harris (1990) determined that

heavier cattle had less tender meat, while Sañudo et al. (2004) reported that heavier cattle produced more tender meat. Meat tenderization is closely associated with degradation of skeletal muscle proteins during aging. One protein that is evaluated as a marker for overall protein degradation is troponin T (Macbride and Parrish, 1977). Specially, the accumulation of the 30 kDa fragment is associated with increases in meat tenderization (Harris et al., 2001), which coincides with the decline in shear force (Contreras-Castillo et al., 2016). More recently, the presence of less HSP70 in meat has been related to a lower meat shear force value (Carvalho et al., 2014). To maintain meat quality HSP27 and HSP70 have been indicated to play a crucial role in the heat shock protein family by influencing meat tenderness during the aging process (Ouali et al., 2006). Therefore, the objective of this study is to evaluate TnT degradation and HSP70 in two muscles from different weight beef carcasses and how they correlate to carcass measurements during slaughter, processing, and aging. We hypothesized that heavy carcasses would have different postmortem protein abundance and degradation when compared with lighter weight carcasses.

Materials and Methods

Experimental design and carcass measurements

Heavy (> 430.9 kg; n = 22), average (351.5 to 385.6 kg; n = 23), and light (< 317.5 kg; n = 20) beef carcasses were randomly selected over 5 nonsequential days at a commercial abattoir. Carcasses at a large packing plant were identified at the end of the slaughter process and before entering the chilling cooler. Carcasses were monitored for temperature and pH changes for 45 min, 4 h, and 24 h. After 4 h and 24 h postmortem, internal temperature and pH decline of the SM and LD between the 12^{th} and 13^{th} rib was measured using a hand-held thermometer (Chromega-Alomega KHSS-18G-RSC12, Omega Engineering Inc., Stamford, CT) and pH probe

(MPI pH meter, Meat Probes Inc., Topeka, KS). The cooler temperature and air velocity were also measured in the coolers for the duration of carcass data collection.

After a 24 h chill, carcass data, including ribeye area, 12th rib fat, and marbling at the 12th rib were evaluated by trained personnel as well as USDA quality and yield grades. After grading, the carcasses were labeled and followed into the fabrication floor. Upon fabrication, primal cuts (IMPS 103 rib and IMPS 168 top round) were transported to the North Dakota State University meat laboratory, where a 2.5 cm steak was collected from each of the ribeye roll and inside round for shear force after 14-days Samples were collected from the cranial end of the ribeye roll and proximal end of the semimembranosus on d-3 and d-14 postmortem. Samples were frozen at -80°C until they were analyzed for TnT expression and its degradation products at d-3 and d-14 aging period as well as expression of HSP 70 at d-3 aging period.

Laboratory analysis

Sarcoplasmic and myofibrillar protein was extracted from frozen meat samples by the procedure of Melody et al. (2004). Approximately 5 g of muscle tissue was minced and homogenized in 1X Post Rigor Extraction Buffer [10 m*M* EDTA; 100 m*M* Tris; 0.1 % (vol/vol) 2-mercaptoethanol; 2 μ*M* E-64; 0.1 mg/mL trypsin inhibitor, 0.4 m*M* phenylmethylsulfonylfluoride] using a Polytron Kinematica (10/15 with controller and PTA 10S generator; Brinkmann, Westbury, NY) until the tissue was ground entirely. The homogenate samples were centrifuged at 21,100 x g at 4°C for 30 minutes (Allegra 25R centrifuge with TA-14-50 rotor, Beckman Coulter, Fullerton, CA). After centrifugation, the sarcoplasmic portion of supernatant placed into a 1.5 mL tubes and stored at -80°C until analysis.

For myofibrillar protein extraction, the protein pellet resulting from the sarcoplasmic protein extraction was submerged in 15 mL of buffer [10 m*M* Sodium phosphate buffer, pH 7.0;

2% (wt/vol) SDS]. Homogenization was performed by using the same procedure as previously used in sarcoplasmic protein extraction. Then samples were centrifuged at 3000 x g (RCF) for 15 minutes at 20°C (Allegra 25R Centrifuge, Beckman Coulter). The supernatant was transferred to 1.5 mL tubes and stored at -80°C until further analysis. For both protein fractions, protein concentration was determined using the Quick Start™ Bradford Protein Assay using Bradford MM (1976).

Gel samples preparation

Gel loading samples were prepared for Troponin T Western analysis by first making x10 dilutions of extracted myofibrillar protein samples in the same buffer as used for extraction of the myofibrillar protein above. These sample dilutions were further diluted in 18 MOhm H_2O to a final concentration of 0.32 µg/µL total protein. One volume of the 0.32 µg/µL sample was combined with 0.5 volumes of modified Wang's gel loading buffer [3 mM EDTA; 3% (wt/vol) SDS; 30% (vol/vol) glycerol; 0.003% (wt/vol) pyronine Y; 30 mM Tris, pH 8.0] (Wang, 1982; Huff-Lonergan et al., 1996) and 0.1 volumes of 2-mecaptoethanol and then mixed, heated at 70°C for 15 minutes, and stored at -80°C until analysis for Troponin T.

Gel loading samples were prepared for HSP70 Western analysis by diluting the extracted sarcoplasmic protein fractions in 18 MOhm H_2O to a final concentration of 3.2 µg/µL total protein. One volume of the 3.2 µg/µL sample was combined with 0.5 volumes of modified Wang's gel loading buffer [3 mM EDTA; 3% (wt/vol) SDS; 30% (vol/vol) glycerol; 0.003% (wt/vol) pyronine; 30 mM Tris, pH 8.0] (Wang, 1982; Huff-Lonergan et al., 1996) and 0.1 volumes of 2-mecaptoethanol and then mixed, heated at 95°C for 10 minutes, and stored at -80°C until analysis for HSP70.

SDS-PAGE electrophoresis

For TnT, proteins (4 µg) were loaded onto a 1.5 mm thick 15% acrylamide separating gels with 5% stacking gels (37.5:1 acrylamide: bis-acrylamide cross linking) and separated by electrophoresis in a running buffer [25 mM Tris, 0.192 M Glycine, 2.0 mM EDTA, 0.1% (wt/vol) SDS] for 2 h 15 min at 150 V. The separating gels contained 0.1% (wt/vol) SDS, 0.05% AMPER, 0.05% vol/vol TEMED, and 0.38 M Tris (pH 8.8). The stacking gels contained 0.125 M Tris (pH 6.8), 0.1% (wt/vol) SDS, 0.075% (wt/vol) AMPER, 0.125% (vol/vol) TEMED.
For HSP 70, proteins (30 µg) were loaded onto 1.5 mm thick 8% separating gels with 5% stacking gels (37.5:1 acrylamide: bis-acrylamide crosslinking) and separated by electrophoresis in a running buffer for 1 h 40 min at 150 V. The separating gels contained 0.1% (wt/vol) SDS, 0.05% AMPER, 0.05% vol/vol TEMED, and 0.38 M Tris (pH 8.8). The stacking gels contained 0.125 M Tris (pH 6.8), 0.1% (wt/vol) SDS, 0.075% (wt/vol) AMPER, 0.125% (vol/vol) TEMED.

Transfer conditions

After electrophoresis, proteins were transferred onto polyvinylidene difluoride membrane by using BioRad Trans-Blot Turbo Transfer System Protocol with BioRad Transfer Packs at a constant voltage of 25 V and 2.5 A for 12 minutes (TnT) and 10 min (HSP 70). Transfer buffer containing 25m*M* Tris, 0.192 *M* Glycine, 2.0 m*M* EDTA, 15% (vol/vol) methanol, and 0.1% (wt/vol) SDS.

Immunoblotting

After transferring protein, membranes were blocked at room temperature in a solution of 5% nonfat dry milk (NFDM) in 1x phosphate-buffered saline (PBS)-0.1% Tween [80 mM sodium phosphate, dibasic; 20 mM sodium phosphate, monobasic; 100 mM sodium chloride

(NaCl), pH 7.4; 0.1% (vol/vol) Tween 20] for 1 h. Upon 1 h blocking, TnT membranes were incubated overnight at 4°C on a rocker with primary antibody mouse anti-rabbit TnT (clone JLT-12, Sigma T 6277, St. Louis, MO) diluted 1:35,000 in PBS-Tween. HSP 70 membranes were placed in PBS-Tween and incubated overnight at 4°C with the primary antibody mouse anti-bovine HSP 70 (Santa Cruz Biotechnology sc-59572) diluted 1:40,000 in PBS-Tween.

After overnight incubation, membranes were warmed at room temperature for 15 minutes and were washed 3 times for 10 minutes each with 1X-PBS 0.1% Tween. After washing, blots were incubated 1 h at room temperature with goat anti-mouse H+L superclonal HRP conjugate (ThermoFisher A28177) diluted at 1:100,000 in PBS-Tween. Blots were washed 3 times for 10 minutes each with 1x PBS-0.1% Tween. ECL prime Western Blotting Detection Reagents (GE Healthcare Life Sciences, Piscataway, NJ) were used to see immunoreactive bands. Chemiluminescence was measured and photo-documented with an Alpha Innotech FluorChem FC2 image analysis system. Densities of immunoreactive bands were normalized relative to a pooled control.

Statistical analysis

All statistical analyses were performed with the MIXED procedure of SAS v.9.4 (SAS Inst., Cary, NC). Fixed effects evaluated were carcass size category (n = 3) as treatment, and replication (n = 3) used for each treatment. Interaction of laboratory sample ID and treatment was tested as a random effect in the TNT and HSP70 protein degradation analysis model in both LD and SM muscles. Model testing was performed using goodness-of-fit statistics (AIC, AICC, or BIC). The random effect of the interaction of ID and treatment was only used in the final model of TNT degradation analysis in SM muscle as the inclusion of random effect improved the model. Least squares means were generated for significant effects and adjusted to control for

experiment-wise error using the Tukey-Kramer method. Pearson correlation coefficients were determined using the *rcorr* function and plotted using the *corrplot* function of R (R Development Core Team, 2020).

Results

Temperature profile

Heavier carcasses showed a slower temperature decline compared to other carcasses in both LD and SM muscles (Figure 2.1 and 2.2). In this case, heavy carcasses had a significantly higher temperature than that of light carcasses LD muscle at 4 hours postmortem (P = 0.02). Also, SM muscle temperature was higher in heavy carcasses compared to light and medium carcasses at 24 hours postmortem ($P < 0.0001$). However, no differences were observed in temperature among three different hot carcass weight groups at other postmortem periods in both muscles ($P > 0.13$).

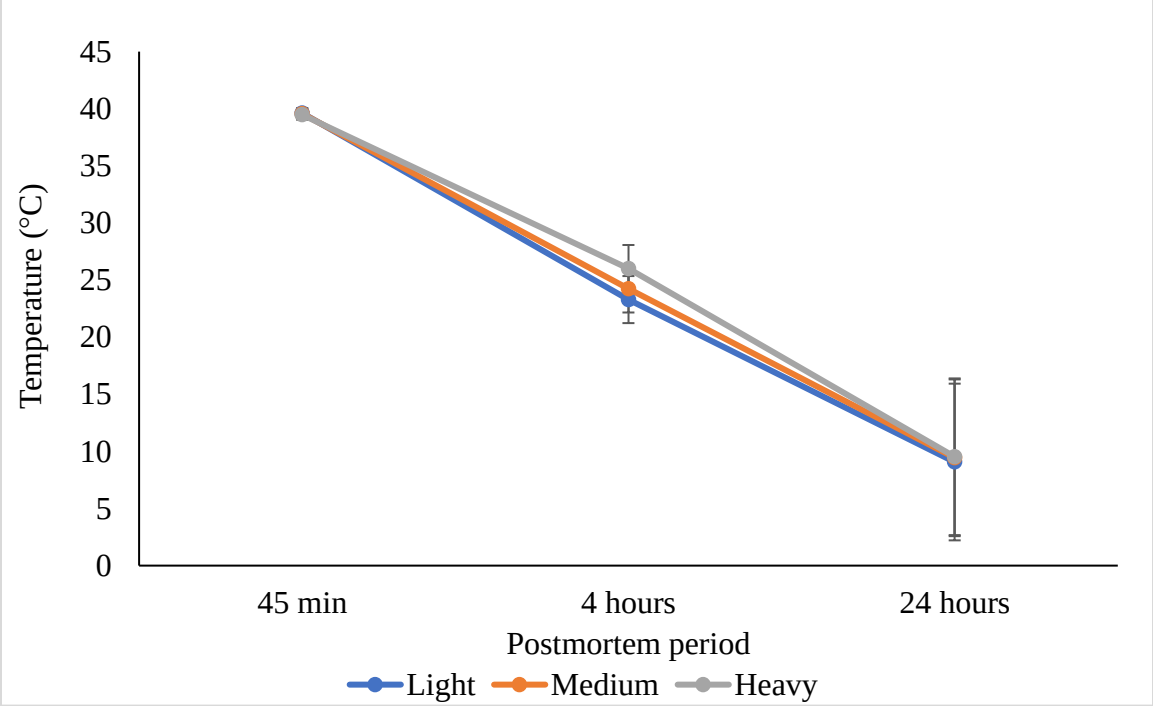

Figure 2.1. Temperature decline over the course of postmortem period for light (< 363 kg), medium (363 – 408 kg), and heavy (> 408 k*g) carcasses in LD muscle [Source: Data from the previously published manuscript of Fevold (2019)]

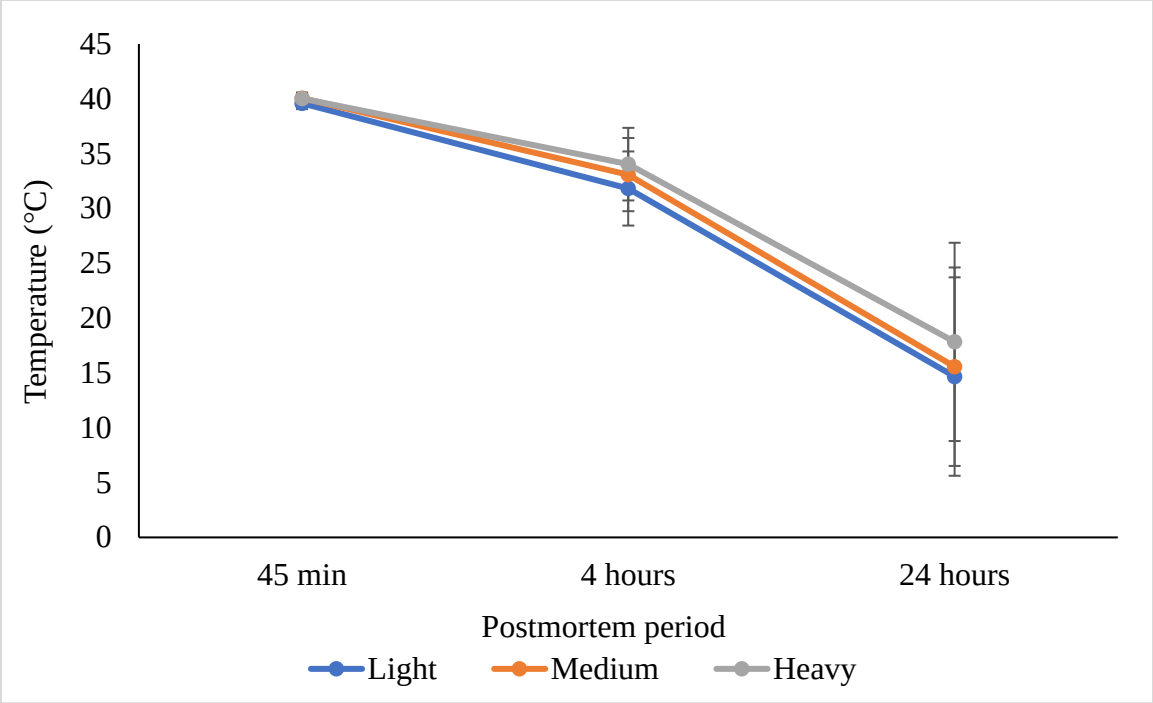

Figure 2.2. Temperature decline over the course of postmortem period for light (< 363 kg), medium (363 – 408 kg), and heavy (> 408 kg) carcasses in SM muscle [Source: Data from the previously published manuscript of Fevold (2019)]

Effect of carcass weight on carcass traits

The hot carcass weight was significantly different among the three treatment groups ($P < 0.0001$; Table 2.1). The LM area was greater in heavy carcasses compared to light and medium carcasses ($P = 0.0002$). The light carcasses had a lower USDA yield grade than the heavy carcasses ($P = 0.04$). No differences were observed among hot carcass weight groups for 12th rib fat thickness, KPH, WBSF for LD and SM, and marbling ($P \geq 0.10$).

The effect of different carcass weight from d-3 and d- 14 aged LD and SM muscle on TnT and HSP70 degradation

There was no influence of carcass weight on TnT degradation at d-3 LD muscle ($P > 0.284$; Table 2.2 and Figure 2.3). However, light carcasses had a greater 30 kDa TnT degradation product than heavy carcasses at d-14 aged LD muscle ($P = 0.007$; Table 2.2 and Figure 2.4). No difference in the degradation product of HSP70 was observed among different carcass weight

groups at d-3 aged LD and SM muscles (Table. 2.2, Table 2.3, Figure 2.6, and Figure 2.7). In d-3 SM muscle, heavy carcasses had less 40 kDa (P = 0.02) and greater 34 kDa (P = 0.03) and 30 kDa (P = 0.03) TnT degradation product than light carcasses (Table. 2.3 and Figure 2.5).

Table 2.1. Least squares mean for beef carcass characteristics of hot carcass weights[1]

Item	Hot Carcass Weights[2]			SEM[3]	*P*-value
	Light	Medium	Heavy		
n	20	19	20		
HCW, kg	337[a]	385[b]	450[c]	3.8	< 0.0001
12[th] Rib fat thickness, cm	1.2	1.3	1.6	0.1	0.12
Longissimus area, cm^2	78.5[a]	83.0[a]	91.1[b]	2.6	0.0002
KPH, %	2.3	2.3	2.3	0.2	0.99
USDA final yield grade[4]	3.1[a]	3.4[ab]	3.7[b]	0.3	0.04
Marbling score[5]	452	458	462	18.8	0.88
WBSF, kg					
Longissimus	2.18	2.19	2.22	0.13	0.97
Semimembranosus	4.27	3.83	3.73	0.22	0.10

[1]Data from the previously published manuscript of Fevold (2019)
[2]Light < 363 kg, Medium 363 – 408 kg, Heavy > 408 kg
[3]Pooled standard errors of the means
[4]USDA Yield Grade determined as 2.5 + (2.5 × 12[th] rib fat thickness, inches) × (0.2 × KPH, %) + (0.0038 × HCW, pounds) – (0.32 × Longissimus muscle area, square inches)
[5]Small = 400, Modest = 500
[a,b,c] Means with similar superscripts within rows are not significantly different (P > 0.05)

The effect of different carcass weight from d-3 and d- 14 aged LD and SM muscle on TnT and HSP70 degradation

There was no influence of carcass weight on TnT degradation at d-3 LD muscle (P > 0.284; Table 2.2 and Figure 2.3). However, light carcasses had a greater 30 kDa TnT degradation product than heavy carcasses at d-14 aged LD muscle (P = 0.007; Table 2.2 and Figure 2.4).

Table 2.2. Least square means ± standard error of the relationship among beef carcass weight, Troponin-T and heat shock protein 70 degradation in *Longissimus dorsi* muscle

Item		Beef carcass weight[1]		
		Average	Heavy	Light
Troponin-T				
D-3				
	40 kDa	1.61 ± 0.17	1.63 ± 0.17	1.70 ± 0.17
	38 kDa	0.70 ± 0.09	0.76 ± 0.10	0.68 ± 0.09
	36 kDa	0.39 ± 0.04	0.35 ± 0.04	0.44 ± 0.04
	34 kDa	0.53 ± 0.07	0.49 ± 0.07	0.59 ± 0.07
	30 kDa	0.18 ± 0.07	0.07 ± 0.07	0.17 ± 0.07
D-14				
	40 kDa	1.37 ± 0.14	1.46 ± 0.14	1.27 ± 0.14
	38 kDa	0.56 ± 0.06	0.55 ± 0.06	0.61 ± 0.06
	36 kDa	0.52 ± 0.04	0.49 ± 0.04	0.57 ± 0.04
	34 kDa	1.29 ± 0.09	1.29 ± 0.09	1.52 ± 0.09
	30 kDa	1.31 ± 0.17[ab]	0.93 ± 0.18[b]	1.70 ± 0.17[a]
Heat shock protein 70				
D-3		1.15 ± 0.08	1.08 ± 0.09	1.03 ± 0.08

[1]Heavy > 430.9 kg, average 351.5 – 385.6 kg, light < 317.5 kg
[ab]LSMeans with similar superscripts within rows are not significantly different ($P > 0.05$)

No difference in the degradation product of HSP70 was observed among different carcass weight groups at d-3 aged LD and SM muscles (Table. 2.2, Table 2.3, Figure 2.6, and Figure 2.7). In d-3 SM muscle, heavy carcasses had less 40 kDa ($P = 0.02$) and greater 34 kDa ($P = 0.03$) and 30 kDa ($P = 0.03$) TnT degradation product than light carcasses (Table. 2.3 and Figure 2.5).

Table 2.3. Least square means ± standard error of means of the relationship among beef carcass weight, Troponin-T and heat shock protein 70 degradation in *Semimembranosus* muscles.

Item		Beef carcass weight[1]		
		Average	Heavy	Light
Troponin-T				
D-3				
	40 kDa	3.09 ± 0.33[ab]	2.65 ± 0.34[b]	4.04 ± 0.33[a]
	38 kDa	1.51 ± 0.17	1.48 ± 0.17	1.77 ± 0.17
	36 kDa	1.05 ± 0.09	1.00 ± 0.09	0.94 ± 0.09
	34 kDa	1.42 ± 0.12[ab]	1.63 ± 0.13[a]	1.16 ± 0.12[b]
	30 kDa	2.44 ± 0.35[ab]	3.10 ± 0.37[a]	1.73 ± 0.35[b]
Heat shock protein 70				
D-3		0.86 ± 0.05	0.90 ± 0.06	0.82 ± 0.05

[1]Heavy > 430.9 kg, average 351.5 - 385.6 kg, light < 317.5 kg
[ab]LSMeans with similar superscripts within rows are not significantly different ($P > 0.05$)

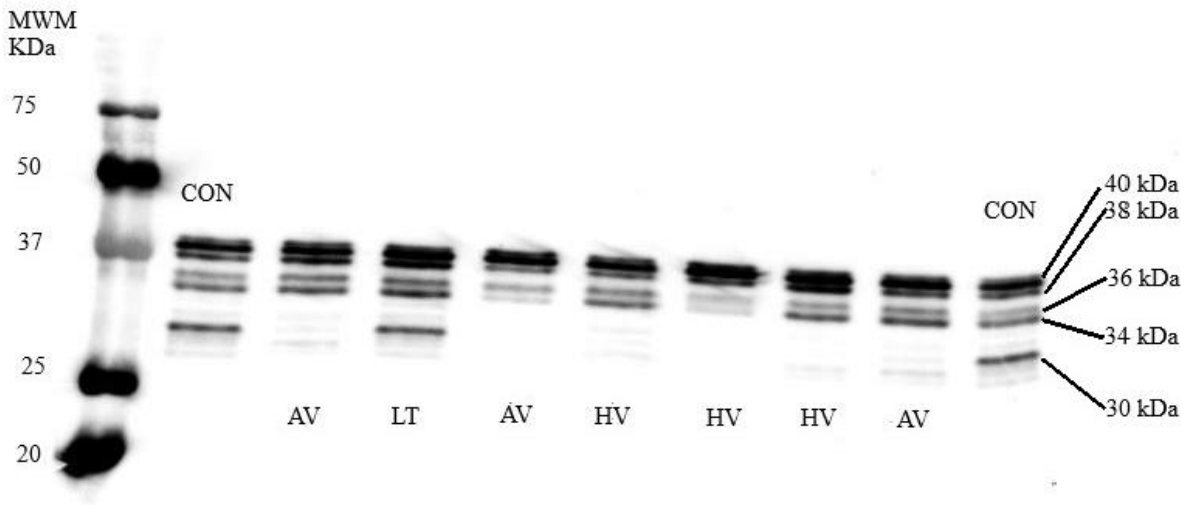

Figure 2.3. Western blot image for TnT degradation at d-3 aged LD muscle from myofibrillar protein sample. Intact band (40 kDa), and degradation products (38 kDa, 36 kDa, 34 kDa, and 30 kDa) were compared to different carcass sizes. A molecular weight marker (MWM) was used to identify the approximate molecular weight of the protein bands.

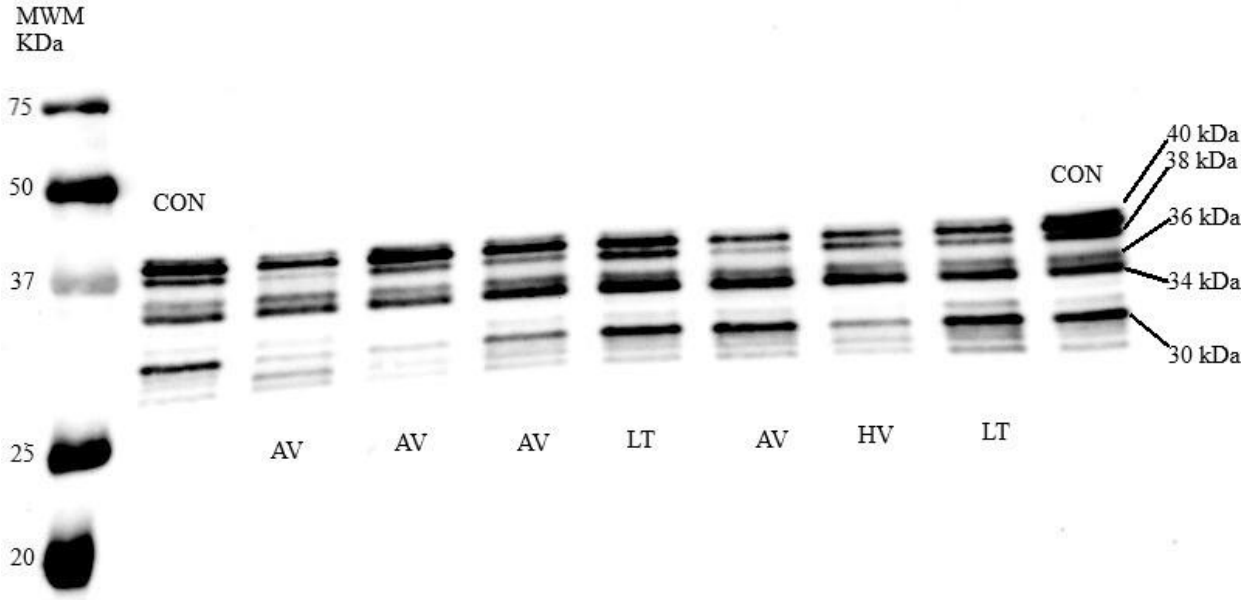

Figure 2.4. Western blot image for TnT degradation at d-14 aged LD muscle from myofibrillar protein sample. Intact band (40 kDa), and degradation products (38 kDa, 36 kDa, 34 kDa, and 30 kDa) were compared to different carcass sizes. A molecular weight marker (MWM) was used to identify the approximate molecular weight of the protein bands.

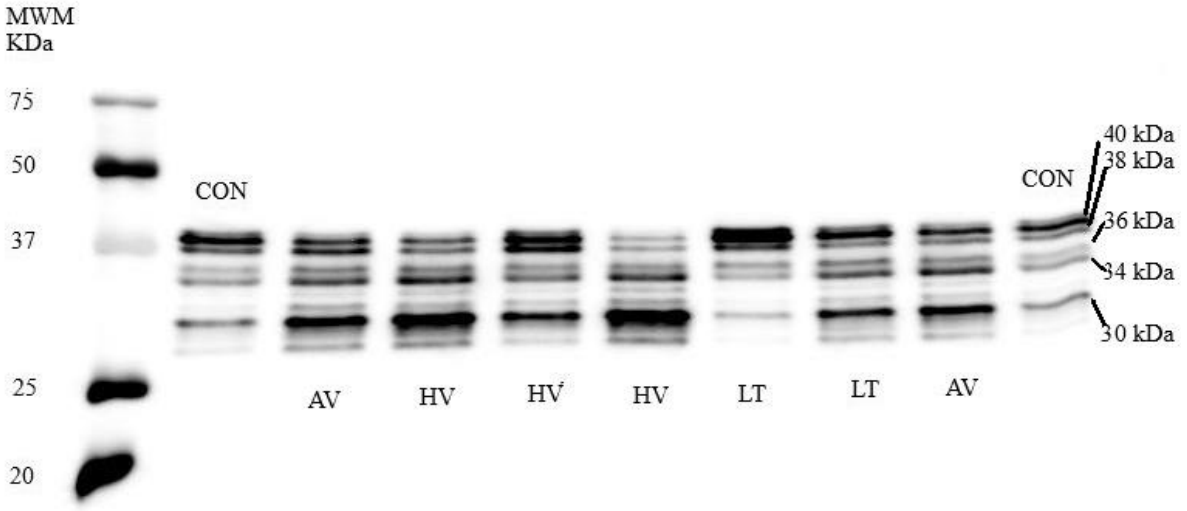

Figure 2.5. Western blot image for TnT degradation at day-3 aged SM muscle from myofibrillar protein sample. Intact band (40 kDa), and degradation products (38 kDa, 36 kDa, 34 kDa and 30 kDa) were compared to different carcass sizes. A molecular weight marker (MWM) was used to identify the approximate molecular weight of the protein bands.

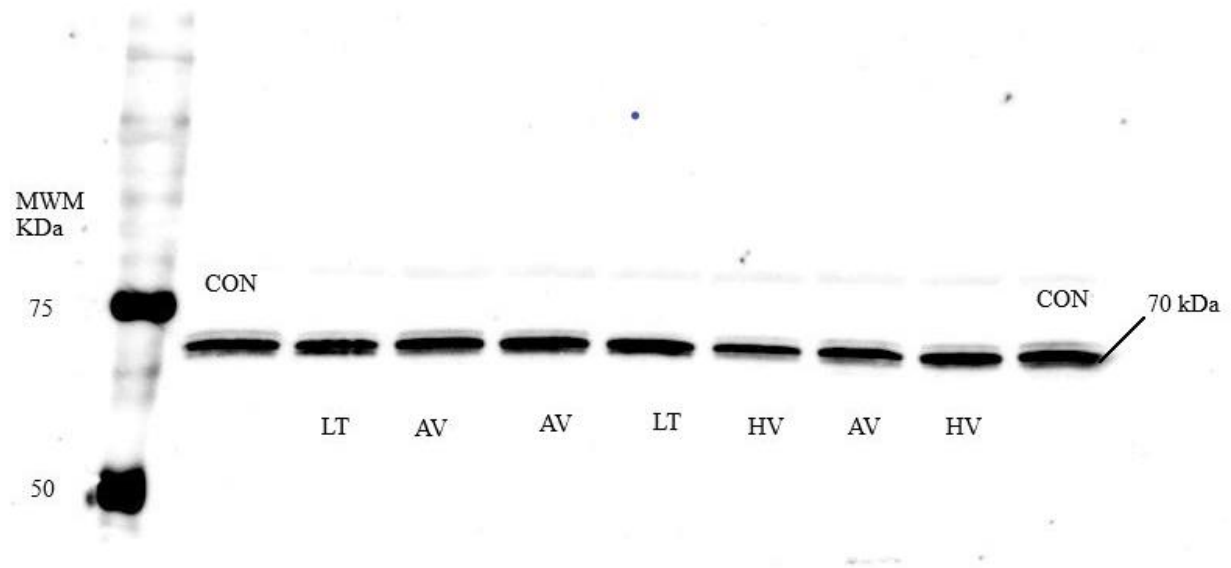

Figure 2.6. Western blot image for HSP70 degradation at d-3 aged SM muscle from myofibrillar protein sample. A molecular weight marker (MWM) was used to identify the approximate molecular weight of the protein bands.

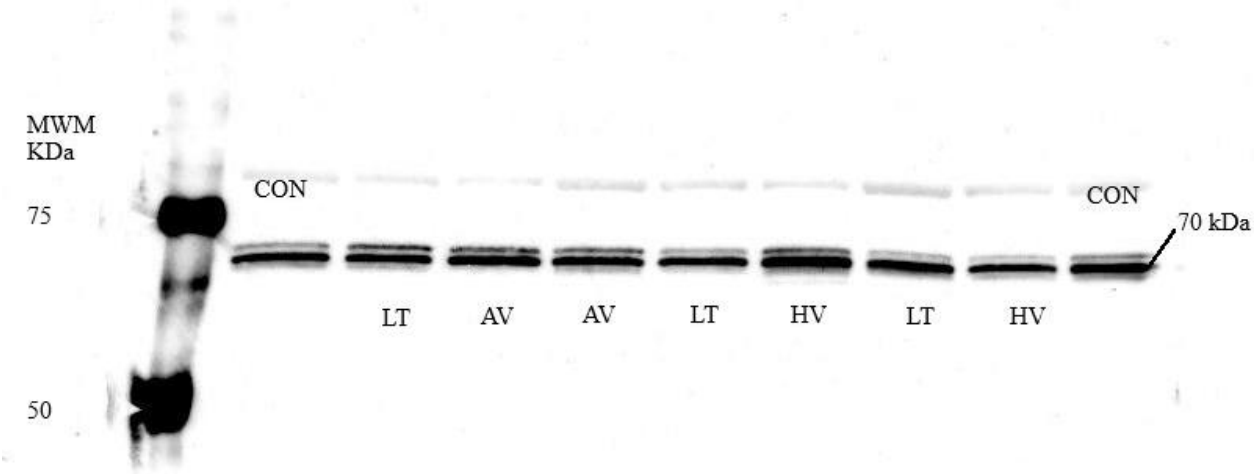

Figure 2.7. Western blot image for HSP70 degradation at d-3 aged LD muscle from myofibrillar protein sample. A molecular weight marker (MWM) was used to identify the approximate molecular weight of the protein bands.

Relationship between carcass weight, pH and temperature at different postmortem period in LD muscle at d-3 and d-14 aging period

In d-14 aged LD muscle, there was a negative correlation between carcass weight and 30 kDa TnT degradation product (r = -0.348, *P* = 0.01; Figure 2.8). There was also a tendency of

positive correlation between 45 min postmortem pH and 30 kDa TnT degradation product at d-14 aged LD muscle (r = 0.25, *P* = 0.06). Additionally, a positive correlation was observed between 4-h postmortem pH and HSP70 at d-3 aged LD muscle (r = 0.455, *P* = 0.03). There was a negative correlation between 4-h postmortem temperature and 30 kDa TnT degradation product at d-14 aged LD muscle (r = -0.287, *P* = 0.03).

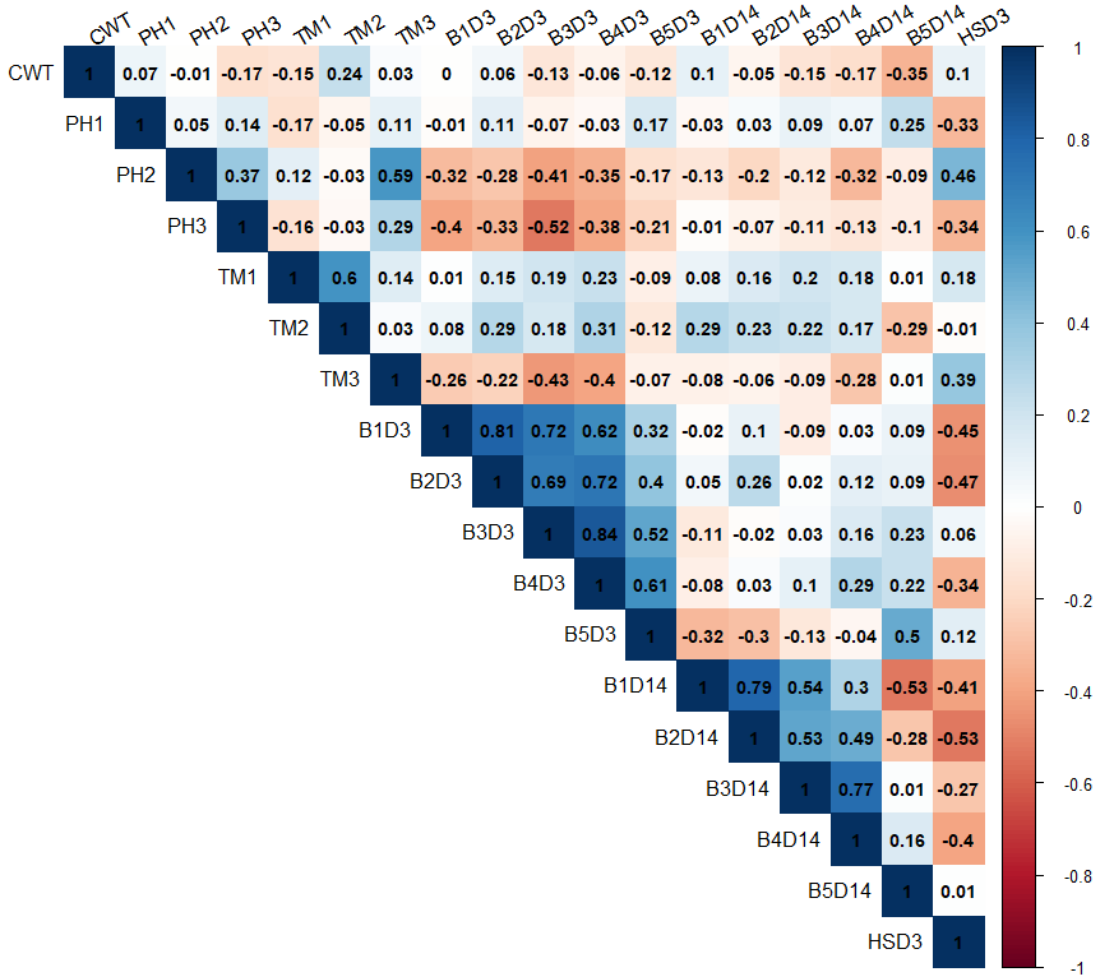

Figure 2.8. Pearson correlation coefficients between carcass weight, pH and temperature at different post-mortem periods, troponin-T (TnT) and heat shock protein 70 (HSP70) degradation product at 3 and 14-day aged LD muscle. Study traits include carcass weight (CWT), pH at 45 min (PH1), pH at 4 hours (PH2), pH at 24 hours (PH3), temperature at 45 min (TM1), temperature at 4 hours (TM2), temperature at 24 hours (TM3), 40 kDa band TnT at D3 (B1D3), 38 kDa band TnT at D3 (B2D3), 36 kDa band TnT at D3 (B3D3), 34 kDa band TnT at D3 (B4D3), 30 kDa band TnT at D3 (B5D3), 40 kDa band TnT at D14 (B1D14), 38 kDa band TnT at D14 (B2D14), 36 kDa band TnT at D14 (B3D14), 34 kDa band TnT at D14 (B4D14), 30 kDa band TnT at D14 (B5D14), and HSP70 at D3 (HSD3)

Relationship between carcass weight, pH and temperature at different postmortem period in SM muscle at d-3 aging period

The carcass weight was positively correlated with 24-h temperature (r = 0.627, *P* = 0.001), and 30 kDa TnT product (r = 0.430, *P* = 0.04); however, it tended to be positively correlated with 45-min temperature (r = 0.380, *P* = 0.07) at d-3 aged SM muscle (Figure 2.9). There was a moderate negative correlation found between 4-h postmortem pH and 30kDa TnT degradation product at 3-d of aging in SM muscle (r = 0.425, *P* = 0.04).

Correlation between carcass data, TnT and HSP products at d-3 and 14 aged LD muscle, and d-3 aged SM muscle

The 30 kDa TnT degradation product was found to be negatively correlated with 12th rib fat thickness (r = -0.321, *P* = 0.003) and ribeye area (r = -0.321, *P* = 0.02) at d-3 aged LD muscle (Figure 2.10). Similarly, the negative correlations were observed between 30 kDa TnT product, and USDA final yield grade (r = -0.352, *P* = 0.007), drip loss percentage (r = -0.270, *P* = 0.04) and shear force value (r = -0.307, *P* = 0.02) at d-14 aged LD muscle. In addition, 12th rib fat thickness was negatively correlated with 36kDa TnT degradation product at 3-d of aging in SM muscle (r = -0.419, *P* = 0.04; Figure 2.11).

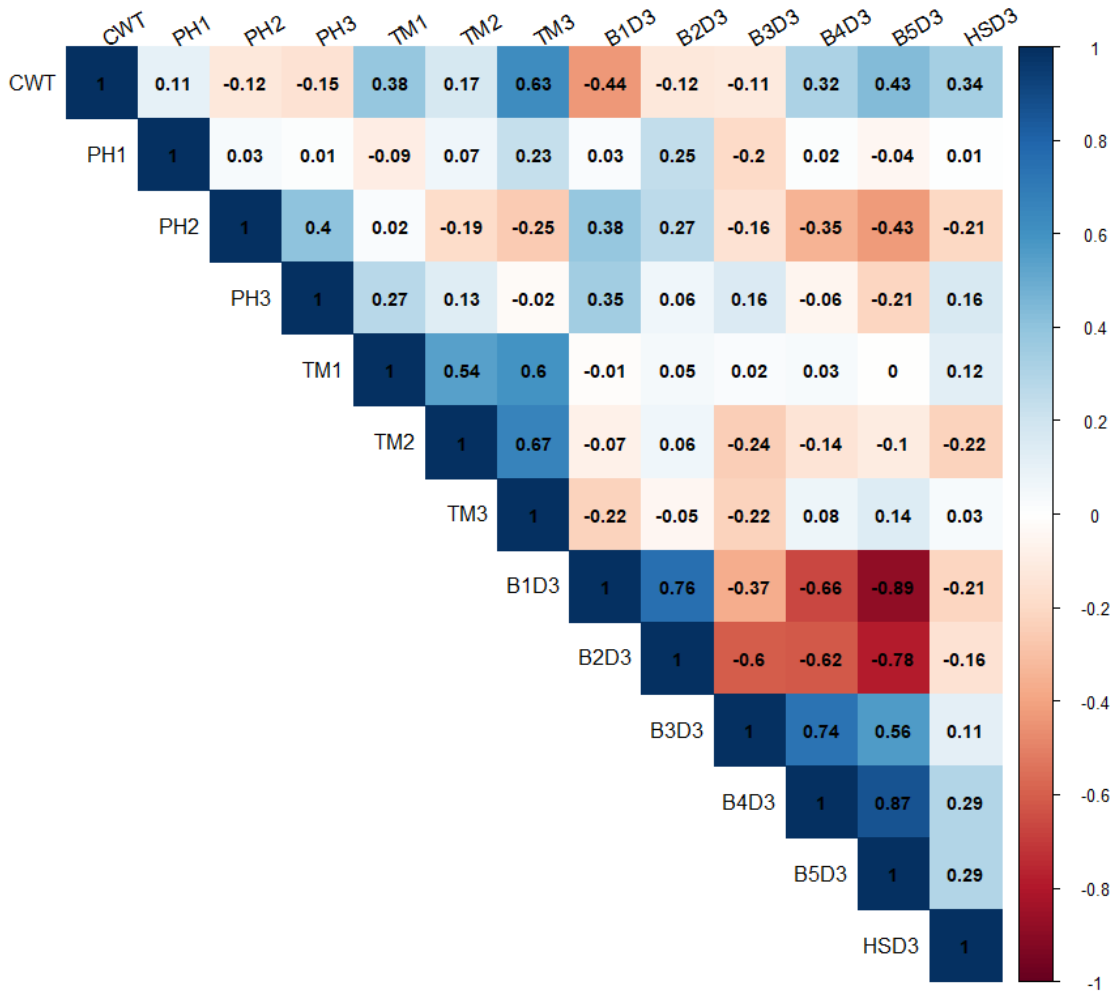

Figure 2.9. Pearson correlation coefficients between carcass weight, pH and temperature at different post-mortem periods, troponin-T (TnT) and heat shock protein 70 (HSP70) degradation product at d-3 aged SM muscle**.** Study traits include carcass weight (CWT), pH at 45 min (PH1), pH at 4 hours (PH2), pH at 24 hours (PH3), temperature at 45 min (TM1), temperature at 4 hours (TM2), temperature at 24 hours (TM3), 40 kDa band TnT at D3 (B1D3), 38 kDa band TnT at D3 (B2D3), 36 kDa band TnT at D3 (B3D3), 34 kDa band TnT at D3 (B4D3), 30 kDa band TnT at D3 (B5D3), HSP70 at D3 (HSD3)

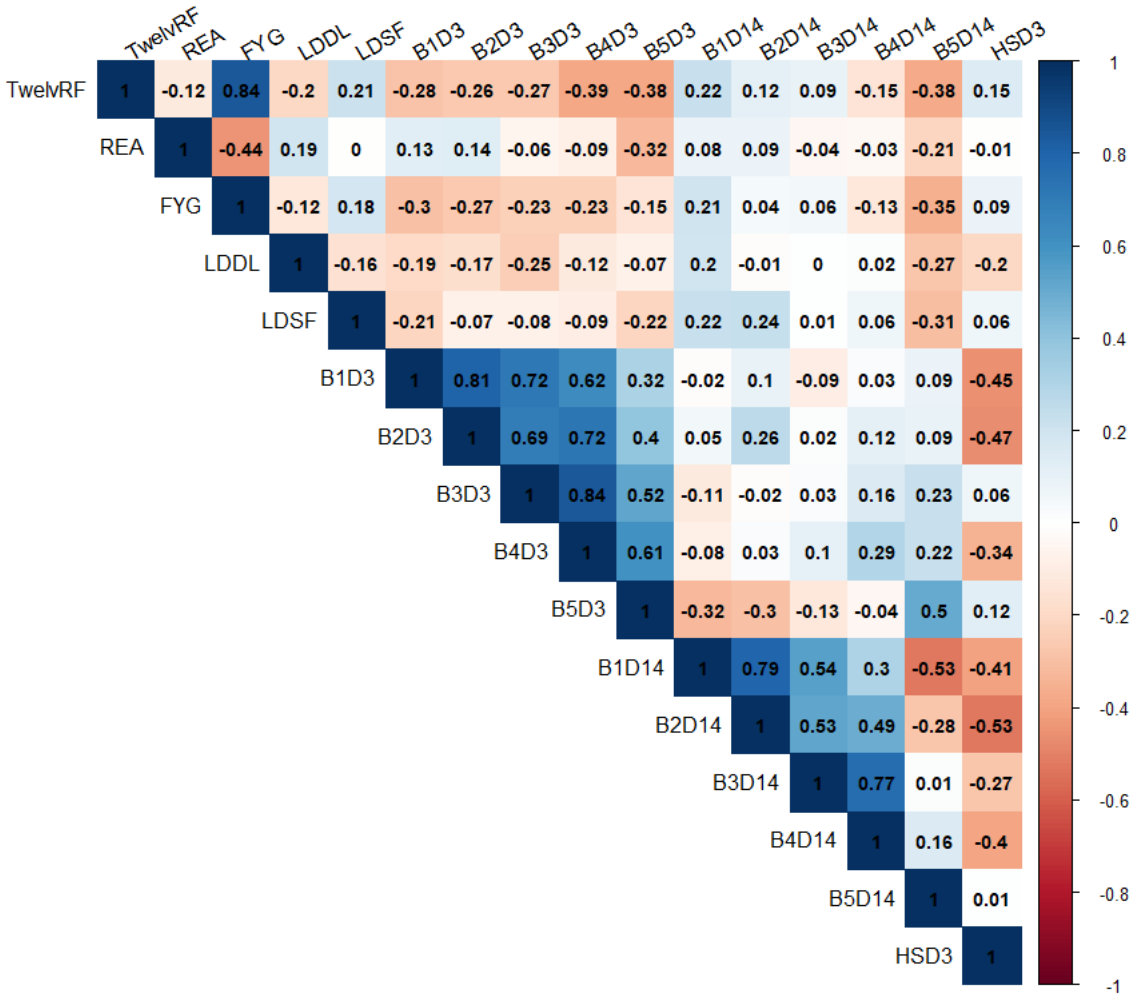

Figure 2.10. Pearson correlation coefficients among carcass data, troponin-T (TnT) and heat shock protein 70 (HSP70) degradation product at d-3 and d-14 aged LD muscle. **S**tudy traits include 12th rib fat thickness (TwelvRF), ribeye area (REA), USDA final yield grade (FYG), drip loss percentage for LD muscle (LDDL), Warner Bratzler shear force for LD muscle (LDSF), 40 kDa band TnT at D3 (B1D3), 38 kDa band TnT at D3 (B2D3), 36 kDa band TnT at D3 (B3D3), 34 kDa band TnT at D3 (B4D3), 30 kDa band TnT at D3 (B5D3), HSP70 at D3 (HSD3)

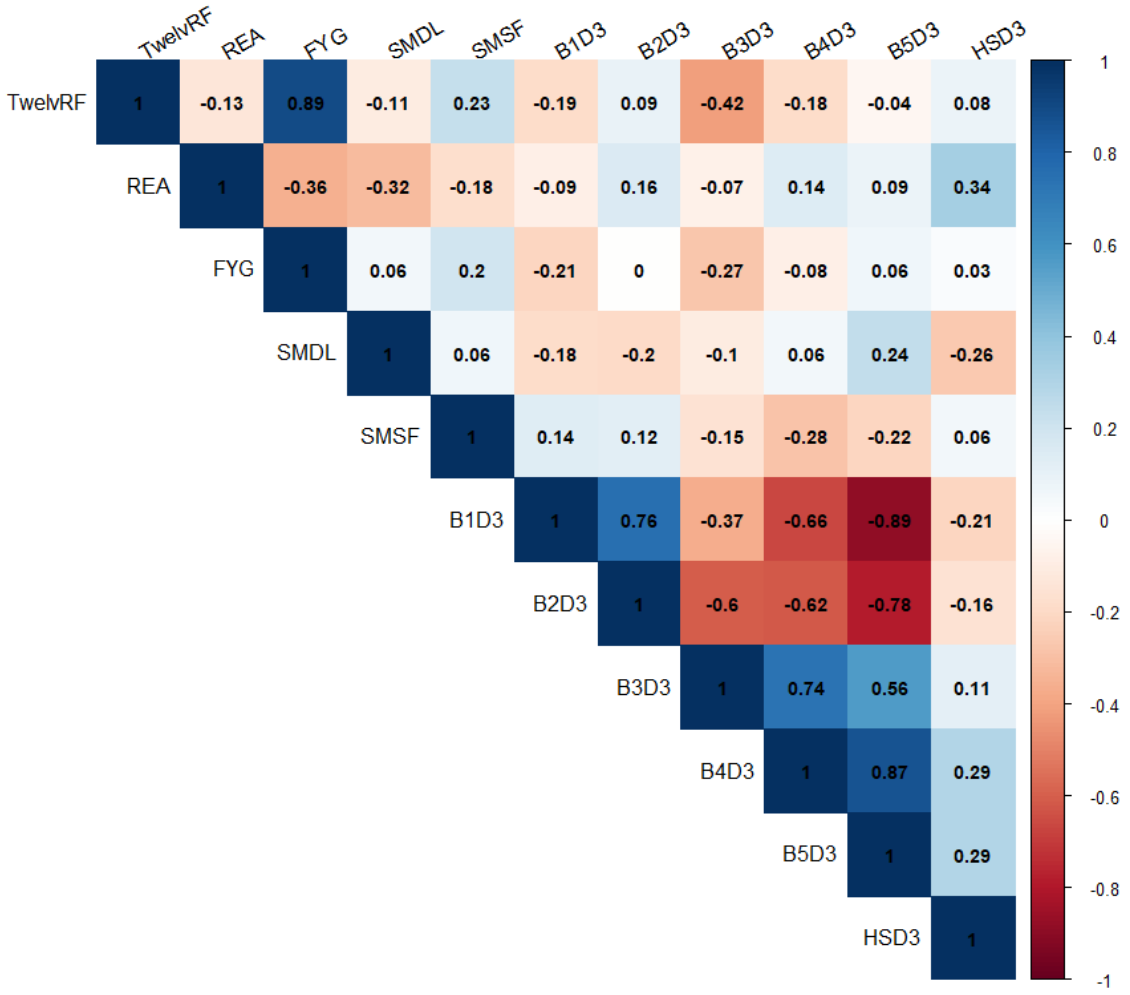

Figure 2.11. Pearson correlation coefficients among carcass data, troponin-T (TnT), and heat shock protein 70 (HSP70) degradation product at d-3 aged SM muscle. **S**tudy traits include 12th rib fat thickness (TwelvRF), ribeye area (REA), USDA final yield grade (FYG), drip loss percentage for SM muscle (SMDL), Warner Bratzler shear force for SM muscle (SMSF), 40 kDa band TnT at D3 (B1D3), 38 kDa band TnT at D3 (B2D3), 36 kDa band TnT at D3 (B3D3), 34 kDa band TnT at D3 (B4D3), 30 kDa band TnT at D3 (B5D3), HSP70 at D3 (HSD3)

Discussion

Meat quality is influenced by skeletal muscle structure and metabolic characteristics, which depend on cellular and molecular events occurring during muscle to meat transformation (Herault et al., 2014). The meat industry has implemented various processing practices, including carcass weight, marbling scores, maturity, fat thickness, etc., which are utilized in the USDA quality and yield grades that help to categorize our beef to ensure eating quality. Many structural

features of muscle have been associated with meat tenderness, including type, amount, and degree of collagen cross-linking, sarcomere lengths, and myofibrillar protein degradation during postmortem aging (Warriss, 2000). Troponin-T is one of the structural myofibrillar proteins that play a significant role in meat tenderness (Macbride and Parrish, 1977). Earlier studies also reported associations of TnT degradation and the appearance of polypeptides in the 30-kDa region with beef tenderness (Macbride and Parrish, 1977; Penny and Dransfield, 1979). Huff-Lonergan et al. (1996) further stated relationships of shear force values with both appearance of the 28- and 30-kDa bands and degradation of intact TnT in myofibrils. They hypothesized that calpain 1 might be partly responsible for TnT degradation and the 28- and 30-kDa polypeptide bands production. Their study also suggested that meat tenderness during postmortem does not result from a single protein degradation; rather, it depends on protein biochemical and structural changes taking place in several essential proteins (Huff-Lonergan et al., 1996a). Troponin-T has been considered a significant biological marker for all protein degradation as the evaluation of TnT's degradation changes is very straightforward (Szalata et al., 2005).

Our study analyzed TnT degradation and HSP70 abundance based on carcass size. In d-3 aged LD muscle, TnT degradation was not influenced by carcass weight; however, light carcasses had greater 30 kDa TnT degradation product compared to heavy carcasses at d-14 aged LD muscle. Our findings are in agreement with some earlier studies where they reported the association of increased TnT degradation with increasing postmortem aging time (Olson et al., 1977; Koohmaraie et al., 1984; Ho et al., 1994). It is generally believed that the endogenous calpain system is one of the significant factors for protein degradation during aging (Zhang, 2009). Under postmortem-like in vitro conditions with low pH and temperature, calpain 1 can act as a catalyst for the degradation of titin, nebulin, filamin, desmin, and TnT into many of the same

degradation products produced in myofibrils of naturally aged muscle samples (Huff-Lonergan et al., 1996a). The calpain 1 becomes associated with the myofibrillar component of skeletal muscle tissue samples with increasing postmortem aging time (Geesink et al., 2000). In contrast, in vitro proteolytic assay displays a rapid reduction of calpain 1 activity at storage period (Boehm et al., 1998; Camou et al., 2007). The combination of low pH and high temperature exhibits early exhaustion of calpain 1 activity during extended aging resulting in tenderization reduction (Dransfield et al., 1992; Simmons et al., 1996). Therefore, changes in muscle pH and temperature at delayed chilling period my influence calpain 1 activity and meat aging. Maddock et al. (2005) observed a greater calpain 1 activity for the samples incubated at a pH of 6.5 compared to those incubated at a pH of 6.0 and 7.5.

The heavy carcasses had a greater degradation of TnT as indicated by the accumulation of the 30 kDa band compared to the lighter carcasses at d-3 aged SM muscle, which was oppositive to the findings observed in LD muscle. These differences suggest that different muscle types may have an impact on TnT degradation. Earlier studies classified both LD and SM muscles as glycolytic; however, these muscles exhibit differences in their myofiber composition (i.e., higher proportion of type IIa myofiber and lower proportion of type IIb myofiber in SM than LD muscle) and metabolic properties (i.e., higher oxidative capacity in SM than LD muscle) (Gentry et al., 2004; Melody et al., 2004; Ruusunen and Puolanne, 2004; Lefaucheur et al., 2011). An earlier study suggested that LD muscle produce more tender meat compared to SM muscle (Dubost et al., 2013). They also reported that the presence of less amount of collagen concentration and collagen cross-links in LD (i.e., positional muscle) becomes meat more tender, whereas their higher amount in SM (i.e., locomotion muscle) leads to the less tender meat. Also, SM muscles need a longer time to dissipate heat than other muscles as they start at a higher

temperature (Hannula and Puolanne, 2004). This agrees with the findings of Djimsa et al. (2018), who reported a slower chilling rate for the round muscle (SM) of heavy weight in comparison to the middle muscle (Psoas major and Longissimus lumborum). Our study also observed higher temperature for SM compared to LD muscle at 3 different postmortem periods. Therefore, muscle location may be related to postmortem temperature decline and, thereby, meat quality.

Low 4-h postmortem temperature and high 45 min postmortem pH were related with high 30 kDa TnT degradation product at d-14 aged LD muscle. In the case of d-3 aged SM muscle, the high 24-h temperature was associated with greater 30 kDa TnT product; however, low 4-h postmortem pH was correlated with high 30 kDa TnT degradation product. The correlation of temperature and pH with TnT degradation product was not consistent at different postmortem period in both muscles. Thus, this TnT degradation might not be temperature or pH dependent.

We observed a higher temperature in heavy carcasses compared to light carcasses at 4-h postmortem for LD muscle and 24-h postmortem for SM muscle. These findings were similar to Agbeniga and Webb (2018), who reported a slower temperature and faster pH decline in heavy carcasses than the lighter carcasses. The higher subcutaneous fat thickness and marbling might act as an insulator, resulting in differences in chilling rates and temperature decline in heavier carcasses (Aalhus et al., 2001; Savell et al., 2005; Juárez et al., 2016). Also, beef carcasses are well known for slowly declining pH traditionally (Pearson and Young, 1989). In beef longissimus muscle, pH between 5.9 and 6.2 at 1.5 h postmortem is desired for optimum tenderness (Hwang and Thompson, 2001b). However, a pH of greater than 5.6 at 3 h postmortem in beef longissimus muscle significantly causes tougher meat due to toughening at high rigor temperature (Pike et al., 1993). An earlier study reported a relationship between muscle pH decline and temperature, where a high-temperature condition causes a rapid rate of energy

substrate utilization resulting in a low pH due to greater accumulation of hydrogen ions (Matarneh et al., 2017).

Our study found a larger LM area for heavy carcasses compared to other carcasses. The current study observed a negative correlation between ribeye area and 30kDa TnT in d-3 aged LD muscle, which indicates the association of larger LD muscle with less proteolysis. Our study also noticed the relationship of lower yield grade (less fat, more muscle), lower drip loss, and lower shear force with higher TnT degradation. These results also mean larger carcasses with greater USDA yield grade are associated with less TnT degradation. However, we have not seen any such relationship in the SM muscle. The shear force of SM muscle may be driven more by connective tissue rather than proteolysis.

Our study did not observe any effect of carcass weight on HSP70 degradation at d-3 aged LD and SM muscles. No relationship was found between shear force and HSP70. The abundance of HSP70 in the early postmortem was positively associated with shear force before and after cooking of LD muscle (Picard et al., 2010). An apoptotic function of the HSP70 has been previously reported by Creagh et al. (2000). Herrera-Mendez et al. (2006) suggested that the tenderization process does not rely on proteolysis only but also apoptosis; therefore, an increased HSP level might reduce tenderness by limiting postmortem proteolytic activity and/or apoptosis process. In another study, Ouali et al. (2006) reported an excess amount of HSPs plays an anti-apoptotic role in the muscle that could inhibit meat aging and consequently negatively impact meat tenderness (Ouali et al., 2006).

Conclusion

Troponin-T degradation was influenced by carcass size in d-14 aged LD muscle and d-3 aged SM muscle but not d-3 aged LD muscles. These findings indicate that LD would have less

TnT protein degradation as the carcasses got larger. The HSP70 was not influenced by carcass size at d-3 aged LD and SM muscles. In the SM, there was an opposite observation where the larger carcasses had more accumulation of the 30 kDa TnT degradation product. However, it seems that the LD and SM muscles and their aging period respond differently to increases in carcass size and impacts on postmortem protein degradation. Therefore, the differences observed in TnT degradation may be related to inherent metabolic factors, including calpain activation rather than processing procedures. This does not seem to be temperature or pH dependent as calculated correlations between temperature, pH, and TnT bands trended similarly in both the LD and SM.

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