EFFECT OF GROWTH PATH ON CARCASS COMPOSITION AND MEATQUALITY

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Animal and Range Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

January, 2013

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ACKNOWLEDGEMENTS

There have been numerous people who have helped me throughout the process of obtaining this degree. I must first thank Dr. Jane Ann Boles for the countless hours of support and guidance she has provided me, and for believing in me. Thanks also to Dr. Michael C. Meyers and Dr. Rachel Endecott for their position on my committee, and for always being willing to help whenever I needed it. I cannot forget to thank K.C. Davis for her patience and help with my statistical analysis.

This project would never have been completed without the work of Audra Ortega, Liz Messier, Carley Stone, and Bailey Engle. Thank you all so very much for your help in the laboratory and for helping to keep me sane during the long days in the lab.

I couldn't have done this without my fellow graduate students, friends, and family who provided the support and laughter that I so dearly needed at times- Devon, Erin, Jaclyn, Katie, Molly, Jake, Kate, Amanda, Tabbethia, Patrick and Merrita (Sean too!), Jim and Lucretia, Erin, Misti, and so many more. Thank you for everything. A huge thank you goes out to my parents who supported me, even when they didn't understand why or what I was doing.

Thank you to the BAIR Ranch Foundation and the Montana Agriculture Experiment Station for partial funding of this project and for the cattle involved in this study. Lastly, thanks to the American Simmental Association and Chappell Feedlot for their cooperation and help.

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ABSTRACT

Reducing variation in tenderness is a goal for the meat industry. The objective of this study was to determine if different growth patterns,not caused by nutrient restriction,impacted carcass characteristics and tenderness. Simmental × Angus steers (n = 18) reared under similar conditions, fed in the same pen at the feedlot, were allocated into fast (n = 9) and slow (n = 9) growing groups based upon four sets of weight data. After slaughter, carcass data were obtained. The striploin was removed, cut into steaks, and aged for 1, 3, 7, 14, and 21 days. For each aging period, one steak per steer underwent evaluation of shear force and MFI. Myofibrils were isolated from aged muscle samples and protein degradation was visualized with the use of SDS-PAGE. Carcass weight ($P< 0.0001$) and LD area ($P = 0.0093$) were significantly affected by growth rate. Shear force ($P = 0.0003$) and MFI ($P< 0.0001$) were significantly affected by length of aging, but not by growth rate ($P\geq 0.3184$); nor was there a significant interaction between growth rate and length of aging ($P\geq 0.6555$). Even though the shear force × growth rate finding was not statistically significant, shear force of steaks from fast-growing animals had a greater reduction in shear force values indicating a difference in early tenderization. A currently unidentified protein with a molecular weight between 130 and 100kDa was seen earlier postmortem in more tender muscle. This degradation was more apparent in muscle from fast growing steers. A 30kDa component also appeared earlier, and at a greater band intensity, in more tender muscle. Degradation of the 30kDacomponent was also more evident in fast growing steers. These results suggest that there is a difference in protein degradation in muscle from animals with differing growth paths. The results of this study suggest that more research is warranted to identify differences in protein degradation due to growth rate and to elucidate the effect of growth rate on variation in tenderness.

INTRODUCTION

The tenderness of a piece of meat has great influence on whether or not it is going to be enjoyed by the consumer. In fact, tenderness of meat is considered to be the foremost quality trait (Lametsch et al., 2004) and, according to Bailey (1972), is likely the most important feature for consumer quality evaluation. Therefore, it comes as no surprise that the tenderness of purchased steaks is extremely critical for consumer satisfaction (Hansen et al., 2006; King et al., 2006; Neely et al., 1998; Shackelford et al., 2001; Torrescano et al., 2003), and has been shown to factor into consumer re-purchasing decisions (Archile-Contreras et al., 2010a). Consumers are more likely to purchase, as well as pay a higher price, for steaks that are guaranteed tender (Feuz et al., 2004; Miller et al., 2001; Shackelford et al 2001). Due to the recent economic downturn, and the increasing price of steaks, meat tenderness is of upmost importance in today's market. However, the United States meat industry has a long history of variation in the steak tenderness(Koohmaraie et al., 1992b; Miller et al., 2001; Morgan et al., 1991; Schroeder and Mark, 2000). This variation in steak tenderness limits steak acceptability and leads to dissatisfaction with the product (Destefanis et al., 2008; Kuber et al., 2004). Therefore, it is widely accepted among producers and retailers that in order to retain customers, it is necessary for the industry to produce a consistently tender product(Koohmaraie et al., 2002;Morgan et al., 1991; Shackelford et al., 2001; Shook et al., 2008; Therkildsen et al., 2008).

According to Klopfenstein et al. 2000, quality of products will affect the future of the United States beef industry. In 2008, there were 2.6 billion pounds of beef produced

with the price per pound averaging $3.69 (NCBA, 2009). Therefore, steak consumption has a large economic impact. According to more recent data from the USDA Economic Research Service (2012), the price of uncooked beef steaks in October, 2012 had reached $6.30 cents per pound as compared to $5.67 in November, 2010, illustrating that there is an even larger economic impact of steak consumption than in the past. Therefore, it is important to produce steaks that are tender, and also, to have consistency in tenderness throughout the industry. It would be beneficial to producers if researchers could identify a method to influence tenderness early on in the production process, as this would allow producers to produce the most tender meat possible and decrease the amount of variability in the marketplace, leading to increased consumer satisfaction. An antemortem method to identify tenderness would also enable the industry to identify less tender product for use in further processing.

The aim of this study was to quantify the effect of growth rate on tenderness as growth rate is a trait that may be determined antemortem, and is easy for the producer to measure. The majority of published research attempting to understand the effect on growth path on tenderness utilizes nutrient restriction to ensure differences in growth. It is not known, however, to what extent nutrient restriction negatively impacts muscle and results from tenderness evaluations. Therefore, this study was conducted to elucidate the effect of natural growth path, with no feed restrictions, on carcass characteristics and meat quality of Angus × Simmental steers in an attempt to identify an antemortem characteristic that may be utilized to enhance steak tenderness and minimize the potential for tenderness variation currently affecting the US marketplace.

LITERATURE REVIEW

Muscle Ultrastructure

Muscle is comprised of long, cylindrical skeletal muscle fibers, known as myofilaments, which are made up of thousands of myofibrils (Greaser, 1991). Myofibrils are made up of repeating sarcomeres (Bailey, 1972), which are the contractile unit of muscle. Sarcomeres are made up of different regions that are identified under phase contrast microscopy. Dark lines that define the length of the sarcomere are known as Z-lines (Greaser, 1991), with the sarcomere stretching from Z-line to Z-line. The I-band area of the sarcomere contains the thin filament (Clark et al., 2002) and is bisected by the Z-line. The A-band region of the sarcomere contains the thick filament and is the region of the sarcomere where the thick and thin filaments interact (Clark et al., 2002). Six thin filaments surround each thick filament (Bailey, 1972), with the thick filament anchoring in the M-line, which are found as a darker line in the middle of the A-band (Greaser, 1991).The H-zone is a lighter area found within the A-band; it is the distance between the ends of the thin filaments (Greaser, 1991). The pseudo H-zone of the sarcomere is along each side of the M-line, and is completely bare of myosin heads (Sawada et al., 1978).

Many of the proteins structures within the sarcomere, such as nebulin, titin, and the thin filaments, are anchored in the Z-line, with the Z-line functioning as the force vector created during contraction (Clark et al., 2002). The Z-line is made up of numerous proteins, with α- actinin, an anti-parallel rod dimer that functions to crosslink actin and titin from adjacent filaments (Clark et al., 2002), occurring at the highest concentration.

Other proteins that make up the Z-line include myopalladin, calcineurin, obscurin, FATZ (γ-filamin/ABP-L, α-actinin and telethonin binding protein of the Z-disc), cypher, Cap-Z, filamin, and myopodin (Clark et al., 2002).

The thin filament consists of multiple f-actin monomers connected in a double stranded filament, which has anα-helical shape. There are seven actin monomers within each half-helical turn (Clark et al., 2002). Along with actin, the thin filament contains tropomyosin and the troponin complex, which function to regulate muscle contraction. Tropomyosin associates with actin and is composed of two α-helical coils, with each tropomyosin dimer spanning seven actin monomers (Clark et al., 2002). The troponin complex - troponin T, I, & C- is bound to the tropomyosin coil (Phillips et al., 1986) with troponins I and T , the globular heads, bound to the troponin T tail (White et al., 1987).

The thick filament is comprised predominantly of myosin (Bailey, 1972; Clark et al., 2002) and the thick filament proteins, such as C-protein, H-protein, and AMP-deaminase (Clark et al., 2002). Myosin molecules are composed of two monomers consisting of two heavy chains and four light chains, the head and the rod (Clark et al., 2002). The head portion of myosin extends from the thick filament, allowing it to interact with actin during muscle contraction (Clarke et al., 2002). As there are six thin filaments around the head region of each thick filament, the myosin heads are offset to ensure that each thin filament may interact with a myosin head also ensures the heads do not interfere with each other. The placement of the myosin heads is very specific; there are myosin heads within the H-zone, but there are no myosin heads protruding into the pseudo H-zone (Sawada et al., 1978). One of the thick filament proteins, C-protein, is

found in a series of transverse stripes around the thick filament (Offer et al., 1972), and potentially functions to link titin and myosin filaments together, and to assist in aligning thick filaments within the A-band (Clark et al., 2002).

Titin, an extremely large protein, forms an uninterrupted filament in sarcomeres, as its molecule spans the A- and I-bands, and the terminal ends from adjacent sarcomeres overlap in the Z-line (Clark et al., 2002). It has a modular structure with very specific repeating motifs, and is elastic in the I-band region (Clark et al., 2002). As mentioned previously, nebulin is anchored in the Z-line, and runs throughout the thin filament, potentially regulating the length of the thin filaments (Clark et al., 2002). Another prominent protein in the sarcomere is desmin, an intermediate filament (Robson et al., 1984; Robson et al., 1991; Clarke et al., 2002). Desmin is associated with the Z line in muscle (Hwan &Bandman, 1989; Robson et al., 1991), where it has been proposed to connect myofibrils together (Robson et al., 1991; Taylor et al., 1995) in a side to side fashion (Clark et al., 2002) and aid in muscle cell stability (Robson et al., 1991)

Muscle Growth

Muscle growth, or protein accretion, occurs when protein synthesis exceeds protein degradation. Protein accretion is thought to occur due to 1) hyperplasia, which is an increase in cell number 2) hypertrophy, which is an increase in cell size and 3) a decrease in protein degradation while the protein synthesis levels remain the same (Koohmaraie et al., 2002). Muscle fiber number is determined prior to birth, but postnatally, the number of nuclei in the skeletal muscles increases during growth (Allen

and Goll, 2003). This occurs via satellite cells, which are also stimulated in adult muscle for regeneration following muscle work and injury (Allen and Goll, 2003).

Satellite cells were first recognized in 1961, when electron micrographs of frog muscle revealed a cell that was "wedged" between the plasma and basement membranes of muscle cells (Mauro, 1961). During postnatal growth, satellite cells fuse and contribute nuclei to muscle fibers, which then leads to an increase in both muscle mass, protein production, and concomitant muscle growth (Moss and Leblond, 1971; Cardasis and Cooper, 1975; Schultz, 1996; Hawke and Garry, 2001). Until satellite cells are stimulated, they are quiescent (McCroskery et al., 2003). Once the satellite cells are stimulated, division and differentiation begins, forming new muscle (Buckingham et al., 2003). Recent research has demonstrated that when satellite cells divide, one daughter cell differentiates and fuses with a muscle cell to donate nuclei to the cell, while the other enters a quiescent stage to be held in reserve to produce more satellite cells and for possible future donation of nuclei if necessary (Schultz, 1966; Beauchamp et al., 2000; Seale and Rudnicki, 2000; Zammit et al., 2004).

Postmortem Protein Breakdown

Postmortem tenderization occurs due to degradation of the proteins in the sarcomere during relaxation of rigor and, therefore, a breakdown of the muscle structure. Although the exact method is not fully understood, it is suspected that three systems work together to accomplish this (Goll et al., 2008). The calpain proteolytic system, consisting of μ calpain, m-calpain, and the inhibitor calpastatin (Murachi, 1983) is the most

probable candidate for initiation of protein degradation (Koohmaraie, 1994), and therefore, postmortem proteolysis. The calpain system will be discussed in depth further on in this review; the remaining proteolytic systems that have been proposed to affect tenderness are the lysosomal system and the proteasome.

The lysosomal system, made up of cathepsins, functions to degrade extracellular proteins during endocytosis (Lecker et al., 1999; Goll et al., 2008), but this system is not thought to initiate postmortem muscle turnover due to the fact that lysosomal enzymes cannot function at the pH of postmortem muscle. Therefore, for degradation to occur, the whole myofibril would have to enter the lysosome, which is not possible due to the large size of the myofibrils (Goll et al., 2008). However, it is possible that once the myofibril has been broken down into filaments, but more likely into fragments of the filaments (Goll et al., 1992) that those pieces could then be broken down further by the lysosomes (Busch et al., 1972; Goll et al., 1992).

The final proteolytic system found in muscle cells is the proteasome complex, which is composed of multiple subunits that form a cylindrical shape and function to degrade abnormal and short-lived proteins (Rivett, 1993). The proteasome complex is involved in the degradation of 80-90% of all cellular proteins (Goll et al., 2008), but just as with the lysosomes, intact myofibrils are too large to enter into the opening of the proteasome structure (Solomon and Goldberg, 1996; Goll et al., 2008). Thick and thin filaments are also too large to enter this opening (Goll et al., 2008), but proteasomes can degrade individual myofibrillar proteins, such as actin, myosin, tropomyosin, and troponin (Solomon and Goldberg, 1996). Once proteins are small enough to enter into the

proteasome complex, they are broken down into amino acids (Solomon and Goldberg, 1996; Goll et al., 1992; Goll et al., 2008). However, actin and myosin are not broken down to a large extent during postmortem protein degradation, which is one reason the proteasome complex is not thought to initiate muscle turnover. Therefore, a proteolytic system other than the lysosomes and proteasomes must initiate protein breakdown (Goll et al., 1992), but it is probable that all three systems work together during postmortem protein degradation.

The Calpain System

Increased postmortem degradation of proteins has been reported to increase tenderness. In fact, Koohmaraie reported that the variation of tenderness among animals of the same age group could be attributed to differences in rate of postmortem protein degradation (Koohmaraie, 1994). Calcium activated factor, or CAF, was originally reported as a potential mechanism of protein breakdown, and it was named as such due to the fact calcium was required to activate the system. Further research indicated CAF was actually a protease system later called calpain. Early research did not differentiate between μ and m-calpain; CAF action described both. CAF was found to remove the Z-line from myofibrils and release α-actinin from the myofibril, which is observed with postmortem degradation and likely related to tenderness development (Busch et al., 1972; Dayton et al., 1975; Cheng and Parrish, 1978; Zeece et al., 1986). CAF also degrades specific proteins related to postmortem breakdown (Dayton et al., 1975; Goll et al., 1992) without degrading actin and myosin, which, as previously stated, have not been shown to

degrade significantly in postmortem muscle. In other words, the proteins that are degraded by CAF are the proteins released during postmortem aging when viewed with sodium dodecyl sulfate polyacrylamide gel electrophoresis, or SDS-PAGE. As Olson et al. (1977) stated, there was a particular protease at work during myofibrillar degradation, because proteolysis was very controlled and specific. Dayton et al (1975) noted that there was a high specificity displayed by CAF, and postulated that CAF could initiate protein turnover. This hypothesis was supported by the observation that both forms of calpain and calpastatin were reported to be intracellular in skeletal muscle, and are found in higher concentrations at the Z-disk than elsewhere in the cell (Kumamota et al., 1992). This is the area where muscle turnover is initiated (Goll et al., 1992).

Although it was known that there were two forms of the CAF enzyme, it was not until 1981 that Dayton et al. discovered that the two forms required different amounts of calcium for activation. One form, μ-calpain, required only 5μM of calcium for activation; m-calpain required 0.1-0.2 mM of calcium. The calcium required to activate the calpains comes from the postmortem release of calcium from the sarcoplasmic reticulum during the conversion of muscle to meat (Aberle et al., 2001).

However, both forms of calpain are not currently thought to be active during postmortem tenderization. The higher calcium requirements for m-calpain, along with the low pH and high ionic strength found in postmortem muscle, are what led some researchers to believe that it is not heavily involved in postmortem protein degradation (Veiseth et al., 2001a; Maddock et al., 2005).Koohmaraie et al. (1987) reported the activity of m-calpain, or CDP-II as it was known at the time, remained relatively stable

throughout 14 days of postmortem storage at 2 ºC, while the activity of CDP-I, or μ-calpain, had an activity profile with a strong similarity to changes in myofibrillar fragmentation, and therefore protein degradation, throughout storage. Accordingly, Veiseth et al. (2001a) reported that there were no changes in the level of m-calpain during 15 days of postmortem storage, suggesting that there is not enough calcium present in postmortem muscle for its activation. This corroborates the study by Morton et al. (1999), which found no changes in m-calpain activity during a 4 day aging period. Additionally, even though the optimum pH for the calpain system is approximately 7.5 (Edmunds et al., 1991), research has shown that μ-calpain is functional at the pH of postmortem muscle (Huff-Lonergan et al., 1996; Maddock et al., 2005), while Camou et al. (2007) found there was very little proteolytic activity of m-calpain at a pH normally found in postmortem muscle. Therefore, as m-calpain cannot be active in the conditions of postmortem muscle, it cannot play a role in postmortem tenderization.

μ-Calpain

μ-calpain has two subunits, with molecular weights of approximately 80kDa and 30kDa (Murachi, 1983; Nakamura et al., 1989;Koohmaraie, 1992a). Once μ-calpain is activated by the calcium release in postmortem muscle, autolysis occurs (Suzuki et al., 1981; Koohmaraie et al., 1987; Koohmaraie, 1992a; Melloni et al.,1992; Geesink and Koohmaraie, 2000) by another μ-calpain molecule (Koohmaraie, 1992a).This causes a decrease in the amount of calcium required to activate μ-calpain, so autolysis continues to occur (Koohmaraie, 1992a; Melloni et al., 1992). Eventually, due to the autolysis, μ-calpain levels are depleted and the enzyme is no longer active (Suzuki et al., 1981;

Koohmaraie, 1992a; Melloni et al., 1992). µ-calpain also causes the degradation of calpastatin; although one researcher reported this does not lead to inactivation of calpastatin, and that the fragments of calpastatin were found to continue their inhibition of µ-calpain (Nakamura et al., 1989), other researchers have reported that it is calpastatin activity that disappears fastest in postmortem muscle during refrigerated storage (Koohmaraie et al., 1987). The discrepancy between these reports likely arises from the fact that the calpastatin in the Nakamura et al. (1989) study utilized purified calpastatin from rabbit muscle directly after slaughter, and was not subjected to storage, while Koohmaraie et al. (1987) removed the calpastatin from muscles directly after slaughter and then also after storage.

Proteins Degraded by µ-Calpain

It has been hypothesized that µ-calpain is the enzyme primarily responsible for postmortem protein degradation (Geesink and Koohmaraie, 2000; Geesink et al., 2005;Geesink et al., 2006)because µ-calpain degrades proteins that degrade during postmortem tenderization, such as tropomyosin (Taylor et al., 1995, Lametsch et al., 2004), troponin-T (Zeece et al., 1986;Taylor et al., 1995; Huff-Lonergan et al., 1996;Lametsch et al., 2004;Geesink et al., 2006), titin (Zeece et al., 1986; Huff-Lonergan et al. ,1996), nebulin (Huff-Lonergan et al., 1996; Geesink et al., 2006), and desmin (Huff-Lonergan et al., 1996; Lametsch et al., 2004; Geesink et al., 2006). Taylor et al. (1995) reported that µ-calpain did not degrade myosin and actin, which are not significantly degraded during postmortem tenderization; this piece of evidence strengthens the hypothesis that µ-calpain is the proteolytic system involved in the

development of tenderness. However, Lametsch et al. (2004) utilized peptide mass mapping through matrix associated laser desorption/ionization time-of-flight mass spectrometry, and reported that µ-calpain does indeed degrade myosin and actin, but that the amount of degradation is so negligible that the degradation would not appear with SDS-PAGE or 2D gel electrophoresis, which may explain why other researcher have reported no degradation of myosin and actin. The reported proteolytic activity of µ-calpain would be irrelevant if the enzyme could not function in the environment of postmortem muscle. Although Zeece et al. (1986) had reported that CAF could cause protein degradation *in vitro* at different temperatures and protein concentrations, including those that were similar to the environment present in postmortem muscle cells, it was not known if µ-calpain would function to degrade myofibrillar proteins in postmortem conditions.

Effect of Postmortem Conditions on the Activity of µ-Calpain

Research has shown that µ-calpain is active at the pH (Huff-Lonergan et al., 1996; Maddock et al., 2005), temperature (Huff-Lonergan et al., 1996), and ionic strength of postmortem muscle (Maddock et al., 2005), lending further strength to the hypothesis that µ-calpain is involved in postmortem protein degradation and therefore, the tenderization process. Morton et al. (1999) found that the more rapid the decline of muscle pH, the faster µ-calpain activity decreased, and the meat from those muscles was more tender. Koohmaraie (1992a) also reported an increase in the rate of autolysis as the pH decreased from 7.0 to 5.8, but noted that the rate of autolysis was also affected by temperature. As

temperatures decreased, the autolytic rate decreased as well. The inactivation of µ-calpain occurred faster when the rate of autolysis increased, and consequently reached its peak at a higher pH and temperature. Therefore, when carcasses are stored in refrigerated temperatures, inactivation of µ-calpain theoretically occurs at a slower rate.

Activity of µ-calpain has been noted as early as 3 hours postmortem (Veiseth et al., 2001a), and although postmortem proteolysis and the related decrease in tenderness have been shown to continue for up to 28 days of ageing (Takahashi, 1996), as will be discussed further on in this review, µ-calpain activity does not continue past 48 hours postmortem (Camou et al., 2007). This suggests that there is still more to be learned about postmortem protein degradation and the enzyme systems responsible for it, as µ-calpain cannot be fully responsible for postmortem degradation after 2-3 days of aging (Camou et al., 2007).

Calpastatin

Both µ- and m-calpain are inhibited by calpastatin (Murachi, 1983). Increased postmortem activity of calpastatin is reported to decrease the activity of µ-calpain and therefore, the amount of postmortem protein degradation and tenderization (Whipple et al., 1990b; Pringle et al., 1997; McDonagh et al., 1999; Morton et al., 1999, Duckett et al., 2000).Researchers have reported that the initial levels of postmortem calpastatin activity were correlated (r=0.83 at 4 days postmortem, r= 0.85 at 14 days postmortem) to increased shear force (Morton et al., 1999), and the ratio between µ-calpain and calpastatin activity have also been shown to affect tenderness (McDonagh et al., 1999; Pringle et al., 1997; Veiseth et al., 2004). An increase in the µ-calpain:calpastatin ratio

was observed concurrently with increased proteolysis (Veiseth et al., 2004), and was positively correlated (r= 0.75) to the myofibrillar fragmentation index and negatively correlated to shear force (r=-0.64, McDonagh et al., 1999) in lambs. In cattle, an increased calpastatin:µ-calpain ratio was found in steaks with increased shear force values and decreased sensory panel tenderness (Pringle et al., 1997). The results from those three studies demonstrated the same concept; increased µ-calpain activity relative to calpastatin activity resulted in more tender meat, while increased calpastatin activity and less µ-calpain activity resulted in tender meat. Furthermore, postmortem calpastatin activity was implicated as a cause of differences in tenderness among *bos taurus* vs. *bos indicus* breeds (Whipple et al., 1990b; Pringle et al., 1997), in decreasing the tenderness of animals with increased muscling, such as callipyge sheep (Duckett et al., 2000), and in decreasing the tenderness of meat from bulls (Morgan et al., 1993a; Morgan et al., 1993b; Woodward et al., 2000)

Proteins and Protein Components Associated with Tenderness

Desmin

Desmin (Koohmaraie, 1994; Huff-Lonergan et al., 1996), titin (Paterson and Parrish, 1986; Huff-Lonergan et al., 1995, 1996), and a troponin-T degradation product, the 30,000 dalton component (Paterson and Parrish, 1986; Huff-Lonergan et al., 1995, 1996) have all been associated with tenderness. Due to its role in muscle stabilization, as discussed in a previous section, numerous researchers suspect that the loss of desmin results in the rest of the myofibrillar breakdown observed during postmortem storage of

muscle, leading to an effect on tenderness (Young et al., 1980; Robson et al., 1981; Koohmaraie, 1994; Taylor et al., 1995; Huff-Lonergan et al., 1996).

As expected for a protein whose degradation is potentially related to tenderness, desmin has been reported to degrade with storage (Young et al., 1980; Hwan and Bandmann,1989;Whipple et al., 1990b; Taylor et al., 1995; Huff-Lonergan et al., 1996; Wheeler et al., 2002; Melody et al., 2004; Rhee et al., 2004; Veiseth et al., 2004), and degrades more rapidly in faster-aging *semimembranosus* muscles (Taylor et al., 1995). Veiseth et al. (2004) found evidence of desmin degradation as early as 9 hours postmortem, and noted that the changes in myofibril fragmentation index were "paralleled" by the degradation of desmin, which is another piece of evidence that desmin proteolysis is related to postmortem tenderization. Some researchers reported that at least half of desmin has undergone proteolysis by 72 hours postmortem (Taylor et al., 1995), and that 94% of desmin is gone by 15 days postmortem (Veiseth et al., 2004), suggesting that desmin degrades quickly and throughout the normal period of tenderization of muscle, and that it might degrade so quickly that it does not play a major role in tenderization after the first 72 hours of aging. However, other researchers have reported that desmin degradation products are still present at 56 days postmortem (Huff-Lonergan et al., 1996), so it is possible that degradation of desmin does continue to occur throughout extended aging periods and could potentially impact the extent of tenderization, although there does not appear to be much research on this topic.

Titin

Titin is a large protein found in striated muscle (Wang et al., 1979); it is one of the largest proteins in the body, and the largest known protein in the sarcomere. A study performed by Lusby et al. (1983) concluded that not only did bovine skeletal muscle contain titin, but that it degraded in postmortem muscle, with the amount of degradation dependent upon both the length of postmortem storage and on the storage temperature. Further research reported that titin is cleaved next to the Z-line during aging (Boyer-Berri and Greaser, 1998), and that μ-calpain degraded titin (Huff-Lonergan et al., 1996).These findings suggested titin might have an impact on tenderness, and Koohmaraie (1994) postulated that the increase in tenderness with postmortem aging is caused by the degradation of titin, which decreases the strength of the myofibril to allow for tenderization. Taylor et al. (1995) reported the majority of the degradation of titin took place at the same time as a large increase in tenderness-between 24 and 72 hours postmortem- lending more evidence to support the theory that titin degradation plays a role in postmortem tenderization of muscle.

Evidence that postmortem aging increased titin degradation is yet another factor relating titin degradation and tenderization; Fritz and Greaser (1991)SDS-PAGE and observed less intact titin and more degradation product bands as aging time of muscle increased. Fritz et al. (1993) further demonstrated that with increased storage time, a second band of degradation products- T2- appeared that was not seen in samples from unaged meat, which was likewise displayed by Huff-Lonergan et al. (1995). Cooking also increased degradation of titin, with a third band appearing in both aged and unaged

samples (Fritz et al., 1993).As the T2 band appeared with the concurrent disappearance of the T1 band, it was concluded that the T2 band was a direct degradation product of T1 (Huff-Lonergan et al., 1995). Although Fritz et al. (1993) demonstrated different degradation patterns of titin with storage and cooking, they did not find a relationship between the amount of titin visualized on SDS-PAGE gels and tenderness. This contradicts many other reports; Paterson and Parrish (1986) found that muscles deemed tender through Warner-Bratzler shear force and sensory evaluations had greater amounts of titin degradation than less tender muscles; the authors noted that the more tender muscles had only the T2 band of titin, while the less tender muscles had the T1 and T2 band, indicating less proteolysis had occurred in those muscles. The difference in the amount of titin degradation has been found to occur as early as 45 minutes post slaughter; not only did the disappearance of the T1 band occur faster in muscles considered to be more tender, but there was also an earlier disappearance of the T2 band, which broke down into smaller polypeptides with a 14-day aging period in the tender muscles (Anderson and Parrish, 1989). Faster degradation of titin in more tender muscles was corroborated in studies by Huff-Lonergan et al. (1995,1996).The age and sex of the animal may also affect titin degradation (Huff-Lonergan et al., 1995), with muscle from older animals and intact bulls and cows showing less proteolysis than younger animals and steers, respectively. This suggests that there is greater protease activity in younger animals.

30,000 Dalton Component

While investigating the breakdown of chicken muscle proteins utilizing gel electrophoresis, Hay et al. (1973) noted the appearance of a band weighing approximately 30,000 daltons. This band only appeared after aging, and was thought to be a component from the breakdown of myosin. However, the appearance of the 30,000 dalton component, or the 30kDa component, after aging was attributed to the breakdown of troponin-T in pork (Penny, 1976), and cattle (Olson et al., 1977). The relationship was confirmed by monoclonal antibody labeling in 1994 by Ho et al. and further corroborated by Huff-Lonergan et al. (1996) with the use of Western blotting.

When visualized using SDS-PAGE, the 30kDa component appears as a band that intensifies with aging (Olson et al., 1977), and the appearance and intensity of the 30kDa component band is linked to sensory and objective values that indicate tenderness, with earlier appearance and greater intensity of the band relating to increased tenderness (MacBride and Parrish, 1977; Olson and Parrish, 1977; Parrish et al., 1981; Paterson and Parrish, 1986; Huff-Lonergan et al., 1995, 1996; Geesink et al., 2001). MacBride and Parrish (1977) used Warner-Bratzler shear force values to group loin steaks as tough or tender, and compared the intensity of the 30kDa component band on SDS-PAGE gels. After 1 day of postmortem storage, the samples from tough steaks did not contain any detectable 30kDacomponent, while the tender steaks did. This discovery led to the terminology of myofibrillar fragmentation tenderness, describing meat which contained the 30kDa component, and where there was myofibril fragmentation “at or near the Z-disk”. Likewise, in 1981, Parrish et al. found the 30kDa component did not appear in the

aged longissimus samples from tough A maturity carcasses, although it did appear in samples from tender A maturity carcasses and both tender and tough E maturity carcasses. This is likely due to connective tissue components causing the toughness of the E maturity carcasses, not the myofibrillar component of tenderness.

The amount of 30kDa component observed in samples increased with increased postmortem storage, and appeared more rapidly in samples from animals classified as tender than in samples from animals classified as tough (Huff-Lonergan et al, 1995; 1996).The designations of tough and tender in both of the aforementioned studies were determined by both shear force value (Huff-Lonergan et al., 1995, 1996) and sensory panel (Huff-Lonergan et al., 1995).White et al. (2006) found steaks with a higher change in Warner-Bratzler shear force, which were held in water baths of 5ºC for 8 hours, had a less dense 30kDa band at 48-hours postmortem than steaks with a lower change in shear force, which were incubated for 8 hours at 25ºC, which means that the muscles with a low change in WBSF began proteolysis sooner. The muscles with the low change in proteolysis were determined by Warner-Bratzler shear force to be more tender; however, the muscles with the highest change in shear force did go through cold shortening, which may negatively affect shear force values. The samples from high change group did have a greater intensity of the 30kDa band at 14 days postmortem. Along with the change in shear force values over a greater period of time, this indicates a delayed proteolysis occurred as compared to the muscles with a low change in shear force. Overall, the results from the White et al. (2006) study indicate that tenderization occurs earlier in certain carcasses than others, based either upon the muscle characteristics themselves or

due to postmortem handling, and that there is potentially little change in proteolysis after that early tenderization; this could be a cause of the variation in tenderness in the marketplace.

Determining Steak Tenderness

Sensory Panels

Currently, the only methods to evaluate meat tenderness are with postmortem testing. These involve both sensory and objective evaluations for tenderness. The sensory assessments of tenderness arise from panels using either trained panelists or untrained consumer panelists, while machinery is used to provide objective measurements of tenderness. Commonly, both trained and untrained panelists assign scores to the steaks based on factors such as tenderness, juiciness, and flavor (Perry et al., 2001). While trained panelists might be more consistent at scoring the traits being investigated, resulting in higher correlations to objective measurements and a lesser variance, they are sometimes more biased than consumer panels (Thompson, 2002). Due to the fact that trained panelists are, by definition, trained to yield consistent scores, there has been speculation that the scores may be affected by this training process (Watson, et al., 2008). However, it is important to note that all aspects of juiciness, flavor, and tenderness interact to influence panel ratings (Silva et al., 1999; Perry et al., 2001; Rhee et al., 2004). Bouton et al. (1973) reported that meat samples with less juiciness due to greater cooking loss, were assessed to be tougher by a trained sensory panel than meat samples with a lesser cooking loss.

Warner-Bratzler Shear Force Measurements

While consumer panels are crucial in order to obtain information about consumer predilections, they tend to be more expensive and time consuming than objective measurements (Perry et al., 2001). One of the commonly used procedures to objectively measure meat tenderness is Warner-Bratzler shear force, which measures the maximum force required to shear through a meat sample with a particular diameter (Bratzler, 1949); the lower the shear force value, the more tender the meat(Bratzler, 1949; Perry et al., 2001).Samples are cooked to a specific internal temperature, allowed to cool, and then cut into cores. Current procedural recommendations include consistent thawing times, using temperature probes to insure that internal steaks temperatures do not differ, cooling steaks prior to coring them to assist in precision of cut, removing cores parallel to the fiber direction of the steak, and utilizing at least six round, connective tissue- and fat-free cores of the same size from each steak (Wheeler and Koohmaraie, 1994; AMSA, 1995; Wheeler et al., 1997). Means of the shear forces of the six samples are used as the "average breaking strength of the cooked muscle from that animal" (Warner, 1929).

The majority of US researchers that analyzes tenderness with Warner-Bratzler shear force use circular cores. However, there is another machine that is used to analyze shear force, the MIRINZ Tenderometer, and the protocol for this equipment calls for the use of square cores (Davey and Gilbert, 1975). The sample is placed into the tenderometer and the shear force required to "bite" across each square sample is measured in kg (Bickerstaffe et al., 2001). Although square cores have been reported to

be less tender than circular cores, with greater shear forces (Davey and Gilbert, 1975; Thiel et al., 1997), they also yielded a more consistent shape and size than the circular cores (Thiel et al., 1997). It is also easier to ensure that samples are removed parallel to the muscle fiber direction when removing square cores (Thiel et al., 1997), which is imperative in order to ensure that the samples are sheared perpendicularly to the fiber direction.

There are still potential drawbacks to establishing validity in reliability, as well as the use of Warner-Bratzler Shear force procedure to identify tenderness. Originally, different establishments utilized different procedures for Warner-Bratzler shear force, resulting in little standardization. Researchers developed recommendations to enhance validity and reliability of the measurements (Wheeler and Koohmaraie, 1994; AMSA, 1995; Wheeler et al., 1997). Derington et al. (2011) reported that there are differences in shear force results from utilizing samples from different parts of the muscle, with the anterior portions of the *longissimus lumborum* yielding more tender meat than the posterior portions. This confirmed findings of Rhee et al. (2004), who concluded that the location where samples were obtained influenced shear force values. in the *psoas major*, *semitendinosus, biceps femoris*, *semimembranosus*, and *rectus femoris*.

It is also important to note that shear force is a one-sided measurement, and cannot account for other characteristics that might affect a consumer's perception of meat tenderness (Perry et al., 2001). Regardless, when compared to other objective measurements, such as compression and cooking loss, Warner-Bratzler shear force was the most useful predictor for sensory tenderness, with both linear and quadratic

relationships observed between WBSF values and sensory panel tenderness scores (Perry et al., 2001) . There have repeatedly been fairly strong correlations drawn between sensory panel evaluations and Warner-Bratzler shear force, ranging from -0.39 to -0.73, despite differences in the scoring and the training of the panels (Bratzler, 1949; Rhee et al., 2004; DeStefanis et al., 2008; Yancey et al., 2010). This suggests that the relationship between sensory panel scores and Warner-Bratzler shear force is strong enough to overcome any panel differences. It has even been reported that consumers will identify tenderness differences revealed by differences in Warner-Bratzler shear force results (Miller et al., 2001; Shackelford et al., 2001). Reports on correlations between sensory evaluations and Warner-Bratzler shear force date as far back as 1929, when Warner reported positive correlation coefficients of 0.6 for beef and lamb and 0.79 for beef ribs between the early "mouse trap" device that evolved into today's Warner-Bratzler shear force machine and the "Home Economics Cooked Meat Committee" grading of meat tenderness. The incongruent findings from the 1929 study may be a result of different evaluation processes in place 80 years ago.

Myofibril Fragmentation

There are numerous factors that positively affect tenderness. One of these factors is an increased amount of myofibril fragmentation, which is measured with the myofibrillar fragmentation index (MFI). Koohmaraie (1994) defined the myofibrillar fragmentation index in aged meat as "the ease of fragmentation of myofibrils under controlled homogenization" and Barkhouse et al. (1996) described the MFI as the "biochemical measure of tenderness based on absorption". The amount of absorption is

related to the size of the myofibrils and pieces of muscle fibers, and has been directly related to tenderness (Olson et al., 1976). Culler et al. (1978) reported a MFI value of 60 equated to very tender meat, and a MFI value below 50 equated to a steak that was not tender. Fragmentation was reported to increase as early as 12 hours postmortem in ovine muscle, and this increased fragmentation was suggested to be due to the activity of μ-calpain (Veiseth et al., 2004). Freezing of the muscle did not appear to affect MFI values (Veiseth et al., 2001b), and one study indicated that the myofibril fragmentation index taken from 24-hour postmortem muscle predicted sensory tenderness better than shear force value of samples from meat that had been aged and cooked (Vestergaard et al., 2000).

Olson and Parrish (1977) reported strong correlations between the shear force values and MFI, as well as MFI and the sensory panel evaluations, with the MFI values increasing with longer aging of meat samples. Correlations reported between the MFI and Warner-Bratzler shear force ranged from -0.65 to -0.93, with correlations ranging from 0.72 to 0.97, between the MFI and sensory tenderness values. The degradation of troponin-T and appearance of the 30,000-dalton component in SDS-PAGE gels was also reported to correspond with high MFI values, with the gels from higher MFI samples displaying an increased intensity of the 30 kDa component band, indicating that the myofibrillar fragmentation index was useful as an indicator of tenderness. Olson and Parrish (1977) also reported that more than 50% of the differences in tenderness between steaks were due to differences in myofibril fragmentation, which was substantiated by Culler et al. (1978).The correlation between MFI values and shear forces has been

supported by other studies (Culler et al., 1978; Crouse and Koohmaraie, 1990; Vestergaard et al., 2000; Chambaz et al., 2003), although Silva et al. (1999) found that the correlation strength decreased as aging time increased.

Factors Affecting Tenderness

Postmortem Aging

As protein degradation has been related to postmortem storage and increasing storage time causes increased tenderness (Paul et al., 1944; Smith et al., 1978; Zeece et al., 1986; Takahashi, 1996; Silva et al., 1999; Gruber et al., 2006; Stolowski et al., 2006; Yancey et al., 2010), the conclusion may be reached that increased tenderness postmortem is related to protein degradation. Protein degradation results in myofibril fragmentation (Olson et al., 1976); it has been shown that the Z-disks degrade as aging time increases (Olson et al., 1976; Young et al., 1980) and individual muscle fibers have been known to become more and more distinct with postmortem aging, with the appearance of contracture nodes as well as "kinks or crinkles"(Paul et al., 1944).Aging has been reported to reduce variation and difference in tenderness of steaks from individual animals and breed groups (Monson et al., 2004; Stolowski et al., 2006). As the difference in postmortem aging time across the country has been implicated as one of the causes of the variation in tenderness (Morgan et al., 1991), the use of sufficient aging periods is considered to be a tool to reduce variation of meat in the marketplace (George et al., 1999; Brooks et al., 2000; Monson et al., 2004; Savell et al., 2006). Postmortem aging usually occurs in temperatures between 1ºC (Paul et al., 1944; Smith et al., 1978)

and 4ºC (Takahashi, 1996) for research purposes. While most researchers agree aging increases tenderness, there is a disparity on the optimal time required. Oftentimes it is recommended that beef carcasses and cuts should be aged for at least 10 to 14 days (Koohmaraie, 1996; Takahashi, 1996) but research disagrees upon whether or not postmortem degradation, and therefore an increase in tenderness with aging, occurs after 21 days postmortem. Takahashi (1996) reported that aging for 28 days gives a considerably more tender carcass. Gruber et al. (2006) suggested the quality grade of the carcass played a role in the amount of tenderization during aging, as the *biceps femoris, infraspinatus, longissimus dorsi, rectus femoris, semimembranosus, spinalis dorsi,* and *tensor fasciae latae* muscles from Select carcasses were found to require at least seven additional days of aging-28 days total- to achieve the same tenderness as the same muscles from Choice carcasses, which required only 21 days. Stolowski et al. (2006) proposed the breed composition of the cattle could potentially be the cause of difference in aging times required to achieve acceptable tenderness. Meat from cattle that were ¾ Angus continued to have postmortem degradation after 28 days of aging, while meat from cattle with a greater *Bos indicus* content did not. This corroborates the report of decreased postmortem protein degradation found in cattle with increased *Bos indicus* content by Whipple et al. (1990b).

Breed Composition

One of the factors reported to have a negative effect on tenderness is breed composition, especially increased *Bos indicus* ancestry. Steaks from British breed cattle have been found to be more tender than steaks from Continental cattle (Chambaz et al.,

2003; Boles et al., 2009). Simmental cattle are a Continental breed, and in a study by Chambaz et al. (2003) steaks from Simmental-sired steers were rated the least tender by a trained sensory panel when compared to steaks from Angus, Charolais, and Limousin steers. The meat was aged for 14 days and the authors speculated that with a shorter aging period, the difference in tenderness between the Simmental steaks and the steaks from the other breeds would have been even greater. There were no statistical differences in Warner-Bratzler shear force between the four breeds; however, the shear force was only measured once, after 14 days of aging, and as aging improves tenderness, any differences that were potentially present could have been masked by the aging. There was a slight numerical increase in shear force for the Simmental steers. The researchers also measured the MFI at 2 and 14 days postmortem, and the muscle from the Simmental steers had significantly lower MFI values at day 2; by 14 days, this difference had disappeared (Chambaz et al., 2003). Although Chambaz et al. (2003) did not report a statistical increase in shear force in Simmental steers, Koch et al. (1976) did report that steaks from Simmental-sired steers had the greatest Warner-Bratzler shear force values and were also rated the lowest in the sensory panel evaluation in a project investigating the effect of six sire breeds on palatability.

The percentage of *Bos indicus* in an animals' breeding has been reported by several researchers to decrease tenderness of steaks. When compared to *Bos taurus* and *Bos taurus* crosses, the *Bos indicus* and *Bos indicus crosses* produce steaks with an increased shear force and decreased sensory tenderness scores (Crouse et al., 1989; Whipple et al., 1990b; Pringle et al., 1997; Barkhouse et al., 1998). Stolowski et al.

(2006) evaluated steaks from steers and heifers of varying levels of Brahman ancestry and found that steaks from half Angus, half Brahman cattle had the highest shear force values after two days of aging, although all differences between the groups disappeared after 14 days of aging. Furthermore, the researchers found meat from half and three quarters Brahman cattle did not continue to tenderize, or continue postmortem protein degradation, after 14 days while meat from the three-quarter Angus cattle did continue to tenderize. This is in disagreement with Schutt et al. (2009), who reported cattle with up to 75% *Bos indicus* could still meet minimum objective and sensory consumer standards for meat quality in Australia, with a shear force under 5.0 kg, an Instron compression score below 2.2 kg, and a "sensory meat quality score of four attributes", or MQ4, greater than 46.5.Crouse et al.(1989) also saw an increase in variation of tenderness of steaks from cattle with a greater *Bos indicus* composition. These researchers suggested the differences in tenderness between steaks from *Bos indicus* and *Bos taurus* cattle were due to decreased myofibril fragmentation in muscle from *Bos indicus* cattle. Furthermore, Whipple et al. (1990b) reported meat from *Bos indicus* cattle possessed a decreased postmortem protein proteolysis due to an increased activity in calcium-dependent protease inhibitor- the former term for calpastatin. Pringle et al. (1997) verified and expanded upon this. These researchers found as the Brahman percentage in cattle increased, there was a decrease in μ-calpain activity in the muscle, along with increased calpastatin activity, and greater calpastatin: μ-calpain ratio, which has been related to decreased tenderness in steaks. As expected from the calpastatin: μ-calpain ratio, there was a linear increase in shear force as the percentage of Brahman increased.

Gender and Sterilization

Gender, sterilization, and age also affect meat tenderness. Although earlier research often reported no significant difference in tenderness between steaks from steers and heifers (Hedrick et al., 1969; Zinn et al., 1970; Prost et al., 1975), Boles et al. (2009) reported that steaks from heifers, sired by both Continental and British breeds, had increased shear force values when compared to steer contemporaries. This agreed with the results from Voisinet et al.(1997), who reported heifers yielded meat that was significantly less tender than steers. When comparing tenderness of steaks from steers to those from both intact and spayed heifers, Choat et al. (2006) reported that steaks from intact heifers had shear force values that were consistently greater than those of steaks from steers at both a common fat thickness and a common marbling score. Furthermore, intact heifers also produced steaks with greater shear force values than spayed heifers at a common fat thickness. This disagrees with the results reported by Field et al. (1996), in which there were no differences in tenderness between steaks from virgin heifers, spayed heifers, and single-calf heifers. When compared to steaks from steers, steaks from bulls have increased calpastatin activity at 24 hours postmortem(Morgan et al., 1993a, 1993b; Woodward et al., 2000), and a decreased rate of protein degradation (Morgan et al., 1993a; Huff-Lonergan et al., 1995; Purchas et al., 2002). These factors were related to increased shear force values (Morgan et al., 1993b; Purchas et al., 2002), decreased sensory tenderness scores (Purchas et al., 2002) and decreased MFIs(Morgan et al., 1993b). Destefanis et al. (2003) evaluated the effect of different times of castration and did not report any significant differences between meat from bulls, late (13 month)

castrated steers, and early (5 month) castrated steers for shear force values, but did report a numerical difference; meat from bulls had higher shear values than meat from early castrated steers, but had lower values than steaks from late castrated animals. The authors did not speculate as to why the late castrated steers had numerically higher shear force values, but intact males had the greatest amount of soluble collagen, which may result in an increased tenderness.

Animal Age

The age of the animal has a large impact on carcass quality and tenderness. When compared to meat from younger animals, meat from 3-, 8-, and 9-year old cows had a decreased rate of postmortem protein degradation, visualized by a later appearance of degradation bands appearing in SDS-PAGE (Huff-Lonergan et al., 1995; Kolczak et al., 2003).Additionally, Bouton et al. (1978) and Shorthose and Harris (1990) found a decrease in both objective and subjective measurements of tenderness as the chronological age of the animal increased. In fact, Shorthose and Harris (1990) described increased chronological age as having a highly significant negative impact on tenderness. Huff-Lonergan et al. (1995) postulated the decrease in tenderness could be associated with decreased calpain activity, or an increased calpastatin activity, resulting in the reported decreased protein degradation. A decrease in soluble collagen due to the increase of insoluble crosslinking has also been shown to occur in older animals (Shimokomaki et al., 1972). The increase in intermolecular crosslinks is caused by a decrease in the rate of collagen turnover (Archile-Contreras,et al., 2010b). Researchers have reported decreased sensory panel scores and increased Warner-Bratzler shear force

values in meat from older animals, whose muscles were comprised of less soluble collagen (Cross et al., 1973). Based upon collagen cross linkages and regardless of the aspects of myofibrillar toughness, Shinkimaki et al. (1972) suggested that the most tender meat would come from animals between 12 and 14 months of age.

Muscle Collagen Content

Collagen, of which there are multiple types, is a robust inextensible fiber (Bailey, 1972) that functions to distribute muscle forces and preserve the structural integrity of myofibrils (Harper, 1999). The endomysium, which is a layer of collagen that surrounds individual cells (Harper, 1999) is composed of type V collagen (Bailey et al., 1979). The perimysium, or the layer of connective tissue that surrounds bundles of muscle fibers (Harper, 1999) is made up of type I and type III collagen (Bailey et al., 1979). Type I collagen forms the epimysium (Bailey et al., 1979), which assists in attaching muscles to bone (Harper, 1999).Both the amount of insoluble collagen (Cross et al., 1973; Light et al., 1985; Torrescano et al., 2003; Riley et al., 2005) and total collagen content (Torrescano et al., 2003; Rhee et al., 2004) have been found to increase shear force values, although the correlations between Warner-Bratzler shear force and total collagen content are not very strong (Purslow, 2005).

It has been hypothesized that differences in tenderness attributed to different muscles are due to collagen content and solubility of collagen within muscles (Rhee et al., 2004; Stolowski et al., 2006), as well as the amount of matrix metalloproteinase 2 (Archile-Contreras et al., 2010b) and oxidative stress (Archile-Contreras and Purslow, 2011). In a study utilizing Brahman steers and heifers, an increase in the amount of

insoluble collagen caused a profound increase in Warner-Bratzler shear force values, with an extra milligram of collagen causing a minimum increase in shear force of 5.0N (Riley et al., 2005). Light et al. (1985) noted an increase in the amount of insoluble crosslinks in three beef muscles classified as tough as compared to more tender muscles; there was also an increased proportion of the perimysium and epimysium in the three tough muscles. The perimysium and epimysium, as previously mentioned, contain type I and type III collagen; Bailey et al. (1979) noted that there appeared to be a relationship between tough muscles and an increased amount of type III collagen, but were unable to explain the relationship. Light et al. (1985) found no correlation between the amount of type III collagen and toughness, but Burson and Hunt (1986) found that there was an increase in the solubility of type I collagen as compared to type III collagen, which could potentially explain what Bailey et al. (1979) found.

Collagen turnover is also relevant to the development of tender meat. As mentioned in the previous section, a decrease in collagen turnover may lead to a decrease in tenderness due to the formation of an increased amount of insoluble cross linkages (Archile-Contreras et al., 2010b). The expression and proliferation of matrix metalloproteinase 2, which may cause a difference in the amount of collagen turnover, was found to differ among three different muscles, and therefore explain why some muscles respond differently to attempts to manipulate tenderness (Archile-Contreras et al., 2010b). Furthermore, it has been suggested that oxidative stress may also negatively affect collagen turnover and solubility, which might again account for differences in tenderness between muscles and animals (Archile-Contreras and Purslow, 2011).There

also may be a link between growth rate and collagen turnover; a decreased growth rate was associated with a decrease in collagen turnover in one study (Calkins et al., 1987). As muscle growth increases, there is also the possibility of an increase in the amount of intramuscular connective tissue, or collagen (Purslow et al., 2012); if there is an increased rate of turnover due to a faster growth rate, the increased amount of collagen will not be as likely to affect tenderness. This is also a potential mechanism to explain why some studies have reported an increased tenderness with increased growth rate.

Conversion of Muscle to Meat

During the conversion of muscle to meat, the pH and temperature of the muscle may vary greatly. After exsanguination, the muscles continue to utilize energy, which can now only be obtained from stored glycogen (Aberle et al., 2001). Due to a loss of oxygen entering the system, the muscle switches to anaerobic metabolism, causing a buildup of lactic acid within the muscle that continues until the glycogen stores are exhausted (Aberle et al., 2001). The lactic acid accumulation decreases the muscle pH, which contributes to changes in tenderness (Aberle et al.,2001). The ultimate pH value and the rate of pH decline are both determined by the amount of glycogen stored in the muscles at death and have been shown to impact tenderness.

Ultimate pH of the Muscle There are differing reports on the effect of the ultimate pH on tenderness. The normal ultimate pH of beef ranges from 5.4-5.7 at 24-hours postmortem (Aberle et al., 2001). Silva et al (1999) reported that a higher ultimate pH, resulting in meat that was considered to be dark, firm, and dry, appeared to produce a

more tender product, even after an aging period of 13 days. In fact, there was a linear relationship between ultimate pH and Warner-Bratzler shear force values; the higher the ultimate pH, the lower the shear force values. Conversely, Veiseth-Kent et al. (2010) observed an increase in Warner-Bratzler shear force values for animals with a greater ultimate pH, which correlates to what Hopkins et al. (2005) found in carcasses from lambs fed a low plane of nutrition. The ultimate pH values in both studies ranged from 5.4 to 5.88 (Hopkins et al., 2005; Veiseth-Kent et al., 2010). In contrast, numerous researchers have reported a curvilinear, not linear, effect of pH on tenderness. In a 1973 study, Bouton et al. studied beef muscle with an ultimate pH range of 5.4 to 7, finding that pH values of 5.8 to 6 resulted in meat with the highest shear forces. Purchas and Aungsupakorn(1993) reported a curvilinear relationship where maximum toughness, as measured by a modified Warner-Bratzler shear force, occurred at an ultimate pH of 6.0-6.2; tenderness increased as the ultimate pH increased from 6.2 to 7.These high pH values give rise to carcasses commonly known as dark cutters, producing dark, firm, and dry meat. One suggested reason for this curvilinear relationship is a reported decrease in the degradation of nebulin in sheep loins with a pH of 5.9-6.3(Wantanabe and Devine, 1994,1995) and titin in sheep loins with a pH of 6.0-6.3 (Wantanabe& Devine, 1995), suggesting that there is a decrease in myofibrillar protein degradation for meat with an ultimate pH up to 6.3. Accordingly, a higher degree of postmortem proteolysis has been demonstrated in animals with an ultimate pH greater than 6.3 (O'Halloran et al., 1994).

Rate of pH Decline The rate at which the pH of a carcass declines has been shown to impact the rate of proteolytic enzyme activation (Dransfield, 1994). Carcasses with an

increased rate of pH decline, which is related to an increased rate of glycolysis, have an earlier activation of proteolytic enzymes, resulting in a faster tenderization than carcasses with a slower rate of pH decline (Dransfield, 1994; O'Halloran et al., 1997).Tornberg et al. (2000) studied nuclear magnetic resonance measurements of *longissimus dorsi* muscles from young bulls and attributed the increased proteolysis to the fact that when there was a rapid pH drop, the cell membranes were destroyed, giving rise to sizeable extracellular volumes. Morton et al. (1999) noted that muscles with a higher pH at three hours postmortem did not tenderize as quickly, and that there was a correlation (r =0.94) between shear force and three-hour pH levels, or, a correlation between shear force and rate of pH decline. This is in agreement with results from Veiseth-Kent et al. (2010), who reported that cattle with a rapid pH drop during the first ten hours postmortem were found to yield meat with increased muscle fiber fractures, leading to increased tenderness, and with White et al. (2006), who noted that a pH_3 (pH at 3 hours postmortem) of 5.9-6.2 consistently produced tender beef.

In contrast, Marsh et al. (1987) noted that muscles that underwent rapid glycolysis yielded the toughest meat as measured by sensory and objective means. The most tender meat was obtained when pH_3 values were intermediate, between 5.9 and 6.3 (Marsh et al., 1987).At first glance, this is dissimilar to the results found by O'Halloran et al. (1997),who reported that fast-glycolysing muscles, with a faster pH drop, exhibited an increased amount and rate of protein proteolysis than slow-glycolysing muscles, resulting in greater tenderness in fast-glycolysing muscle. However, the fast-glycolysing muscle reached a pH_3 of 5.94, which puts the muscles into the same category as the intermediate-

glycolysing muscle of Marsh et al. (1987). Wahlgren et al. (1997) utilized a set pH measurement of 5.6 for their research on the effect of rate of pH decline on tenderness, with the muscles being categorized depending upon how long it took to reach that pH. The authors reported that a "medium pH-time course", where the pH of 5.6 was reached at 10hours postmortem, was significantly more tender based upon sensory ratings and shear force values.

It is apparent that the description of the rate of pH decline varies between studies; therefore, it is the pH values themselves that are important, rather than the category that they are put in. It is also important to note that oftentimes electrical stimulation of carcasses, which is utilized to increase the rate of glycolysis and pH decline and consequently decrease the length of rigor mortis (Savell et al., 2005), is used in studies to simulate specific rates of pH decline. O'Halloran et al. (1997) did not use electrical stimulation, and this is a potential reason why the pH values in their fast-glycolysing muscle were similar to the values obtained by Marsh et al. (1987) and Wahlgren et al. (1997) from their intermediate-glycolysing muscle.

Postmortem Muscle Temperature Postmortem temperature affects muscle in two different ways. Higher temperatures increase the glycolytic rate of muscle (Dransfield, 1994; Thompson et al., 2006), which may cause an increased rate of pH decline and an increase in tenderness as described in the previous paragraph. Researchers often utilize chilling temperature as a method to manipulate the rate of pH decline; as a consequence, many of the results of chilling temperature on the tenderness of meat are also related to the rate of pH decline, making it difficult to separate the effects. It has been hypothesized

that lysosomal enzymatic activity is increased when carcasses are held at higher temperatures due to the low pH produced, which creates an environment that is favorable for the enzymes, and also by increasing the time available for the enzymes to function (Moeller et al., 1976).King et al. (2003) found that excised muscles placed into an ice bath to induce rapid chilling had numerically greater pH values up to 16 hours postmortem, increased Warner-Bratzler shear force values, and also significantly less proteolysis of desmin after 24 hours of aging when compared to muscles from carcass that were chilled at 0º C. The numerically higher pH values and increased toughness of the excised, rapidly chilled muscles support the theory that lower pH values do increase the proteolytic enzyme activity in muscle. However, it is important to note that there was only a difference in desmin proteolysis between chilling regimes at 24 hours; there was no difference found after 14 days of aging. Hwang and Thompson (2001) measured the pH of beef *m. longissimus thoracis et lumborum* at 1.5 hours postmortem, and found that although the beef sides that were chilled slowly (held at 18ºC for 2 hours, then put in a 6ºC cooler for 7-11hours) experienced a faster rate of glycolysis and had lower Warner-Bratzler shear force values at 1 day, the meat did not tenderize much during aging, which the authors postulated could be due to an "early exhaustion" of µ-calpain. Slow-chilled muscle with a faster glycolytic rate was found to have decreased amounts of µ-calpain as compared to muscle that was rapidly chilled. As a result, the most tender meat after 14 days of aging was meat that had gone through an intermediate rate of pH decline, with a pH of 5.9-6.2 at 1.5hours postmortem. Devine et al. (2002) allowed electrically stimulated sheep *m. longissimus thoracis et lumborum* to enter rigor at 18˚ and 35˚C, and

their results indicated a decrease in the rate and extent of tenderization in the meat that entered rigor at 35˚C, similar to the results of Hwang and Thompson (2001). Devine et al. (1999) reported the extent of aging was reduced in beef that entered rigor at 20˚, 25˚, 30˚, and 35˚C. The 35˚C meat did have a significantly higher ultimate pH at 24 hours postrigor (Devine et al., 2002), so the decreased proteolysis supports the results by Moeller et al. (1976), King et al. (2003), and Tornberg et al. (2000), which indicated increased proteolysis with lower pH values. Conversely, Marsh et al. (1981) reported an increased sensory tenderness when carcasses were held at 37˚C for the first three hours after slaughter; while no objective measurements or measurements of postmortem protein breakdown were performed, the authors attributed this tenderness to an increased proteolysis that occurred at high temperatures.

Cold Shortening The second way muscle is affected by postmortem temperature is by the temperature influencing the amount of muscle shortening that occurs (Locker and Hagyard, 1963; Bailey, 1972;Dransfield, 1994; Savell et al., 2005; Thompson et al. 2006; White et al., 2006). A faster chilling rate has long been known to cause a decrease in tenderness (Ramsbottom and Strandine, 1949; Locker, 1960), which was attributed to the amount of muscle contraction (Locker, 1960; White et al., 2006). This phenomenon is known as cold shortening. However, there are differing opinions on the temperature that muscle should reach prior to rigor in order to prevent cold shortening. According to Dransfield (1994), cold shortening may occur when the temperature is below 10 ˚C and the muscle pH is greater than 6.2.Savell et al. (2005) suggested that attempts should be made to prevent muscle temperatures from dropping below 10ºC until the pH has reached

6.2 in order to avoid cold shortening. Bailey (1972) reported that extreme shortening occurred when the muscle temperature was below 10ºC at the time rigor mortis set in, but there was minimum shortening when rigor mortis occurred at 15ºC. Devine et al. (1999) also reported minimal shortening at 15ºC. Locker and Hagyard (1963) found that temperatures between 14˚ and 19ºC produced the least amount of shortening, with temperatures lower than that causing an immediate shortening of muscle fibers. While higher conditioning temperatures may cause an increase in the rate of glycolysis and therefore improved tenderness, it is also possible for higher temperatures to cause a less extreme muscle contraction, called heat, or rigor, shortening, which will negatively affect tenderness (Thompson et al., 2006). This shortening is due to the increased glycolytic response, causing a release of calcium and subsequent contraction (Thompson et al., 2006). While not as severe as the shortening that occurred in cold temperatures, both Locker and Hagyard (1963) and Bailey (1972) noted a degree of shortening when rigor occurred at temperatures above 19ºC (Locker and Hagyard, 1963) and 25ºC (Bailey, 1972).

Growth Promoting Implants

There are differing opinions on the effect of growth promoting implants on the tenderness of meat. While Platter et al. (2003) and Reiling and Johnson (2003)reported that multiple implants decreased tenderness and Boles et al. (2009) reported a decrease in tenderness with only one implant, Schoonmaker et al., (2001) found that neither the Warner-Bratzler shear force nor sensory characteristics of the longissimus from implanted steers were significantly impacted. There was a slight numeric difference

between the groups for shear force, however, with the aggressively implanted steers having an average shear force of 5.0 kg and the non-aggressively implanted steers having an average shear force of 4.9 kg. Interestingly, Kerth et al. (2003) reported that although sensory panels could not differentiate between implanted and non-implanted steers, there was an increase in Warner-Bratzler shear force between steaks from heifers that had been implanted twice and control heifers that had not been implanted. The study also measured rates of protein synthesis and degradation, and implanted heifers were found to have an increased amount of protein synthesis along with a decrease in the amount of protein degradation. Conversely, Roeber et al. (2000) reported that while the consumer panel did report a decrease in tenderness and overall desirability in implanted steers, there was no significant difference for implanted animals in Warner-Bratzler shear force. Numerically, however, there were lower shear forces for unimplanted steers, which matches the report from the consumer panel. To further confuse the issue, Barham et al. (2003) observed that implanting *Bos* indicus-cross cattle had no effect on Warner-Bratzler shear force values and consumer sensory panel ratings after an aging period, but that trained sensory panelists ranked implanted steers lower, and therefore less tender, even after a 14-day aging period. Igo et al. (2011) noted that differences in sensory tenderness evaluations were based upon both quality score and amount of postmortem aging; there were no reported differences for tenderness in Choice steaks, but 14-day aged Select steaks from implanted animals were ranked lower than steaks from non-implanted animals. However, after another week of aging, while overall likability was still higher for non-implanted animals, there were no significant differences in tenderness. This contrasts with the

findings of Boles et al. (2009), who noted that there were no improvements in tenderness after 14 days of aging. That study also revealed that although all implanted animals had an increase in shear force values, cattle sired by a British breed had less of an increase than those sired by Continental cattle; as mentioned previously, heifers of both breeds also had decreased tenderness. Therefore, one of the possible reasons for the discrepancies among the available information regarding implants and tenderness could be the different breed and gender compositions of animals in the studies (Boles et al., 2009).

Growth Rate and Tenderness

The effect of growth rate on tenderness is a highly researched and contested topic. According to Koohmaraie et al.(2002), a decrease in protein degradation, and therefore an increase in muscle growth, may have negative effects on the tenderness of those muscles after they have become meat. This viewpoint is supported by the study that Duckett et al. (2000) performed on the calpastatin activity in callipyge lambs. Callipyge is an increase in postnatal muscle hypertrophy in lambs. There is an increase in calpastatin activity in lambs with the callipyge mutation, and chops from those lambs also had a greater shear force value (Duckett et al., 2000). An increase in calpastatin activity, as found in the callipyge lamb study, has been theorized to lead to a decrease in the activity of μ-calpain, which diminishes the amount of proteolysis that occurs postmortem. In a 2009 study by Boles et al., animals of Continental descent had larger ribeye areas than animals of British descent, and also produced steaks with greater shear force values.

However, calpastatin activity levels were not measured in the Boles et al. study, so it was not possible to ascertain if the decrease in tenderness with muscling is derived from calpastatin.

Other studies have shown that the growth rate of cattle does not affect tenderness characteristics. Loken et al. (2009) restricted nutrition during backgrounding for one group of steers, and after the cattle were harvested, there was no difference between the groups for hot carcass weight, Warner-Bratzler shear force, or sensory tenderness scores. Allingham et al.(1998)also saw no effect on Warner-Bratzler peak force when nutrient restriction was used to reduce growth rate; this study involved three groups of Brahman-cross cattle, two of which were nutritionally restricted and then fed for compensatory growth. While investigating the difference between fast-growing, concentrate-fed cattle and slower growing, grass-fed cattle, Archile-Contreras et al. (2010a) did not see a difference in shear force values between groups. In agreement with these prior studies, Greenwood et al. (2006) did not find a difference in shear force due to differences in the birth weight and pre-weaning growth rate of Piedmontese and Waygu crosses. There was also no difference found between shear force values and sensory panel tenderness evaluations between bulls of different growth paths in a 1987 study by Calkins et al. Interestingly, Therkildsen et al. (2008) did not find a difference in objective tests of tenderness, but sensory panelists did rate calves who had been fed *ad libitum* to be more tender at two different aging periods.

There are also multiple reports of an increased growth rate positively affecting tenderness in both lambs and cattle. Thatcher and Gaunt (2002) and Hopkins et al. (2005)

both reported that lambs being fed on a low plane of nutrition had tougher meat than lambs with access to better nutrition, which resulted in faster growth rate. Renand et al. (2001) found a trend for more tender meat, as assessed by a sensory panel, associated with bulls that grew faster than their counterparts that had access to the same diet. Perry and Thompson (2005) noted an increase in palatability based upon sensory (MQ4)ratings in faster growing animals when animals within a contemporary group were assessed, rather than contemporary groups as a whole. The authors suggested this occurred due to animal expressing their "genetic potential for growth" within the group. A compensatory feeding strategy for bulls failed to produce more tender meat in the *m. longissimus dorsi* and *m. supraspinatus* than meat from bulls that had been fed *ad libitum* throughout their lifetime (Hansen et al., 2006).Tenderness was measured by utilizing a sensory panel for both the *longissimus dorsi* and *supraspinatus,* and Warner-Bratzler shear force was performed on steaks from the *longissimus dorsi.* Cattle fed to experience compensatory gain had a significantly higher maximum value (N) for WBSF, while the sensory panel preferences for overall texture, tenderness, and overall preference were correlated to bulls fed *ad libitum.* Interestingly, the opposite results were reported for the *m. semimembranosus*; compensatory feeding was positively correlated with the aforementioned sensory panel results; this led the authors to speculate that different muscles differ in their responses to compensatory growth. In a study that compared nutritionally-restricted steers with slow-growing and fast-growing steers, Tomkins et al. (2005) did not find a significant difference in peak shear force values between the groups, but in both the *m. longissimus dorsi* and *m. semitendinosus,* the steers that were

designated as rapid growth had the lowest numerical peak force values. Fishell et al. (1985) did not find any difference in myofibril fragmentation indexes between nutrient-restricted cattle and their faster-growing counterparts, and therefore attributed the increased tenderness of the faster-growing cattle, as shown by both shear force and sensory scores, to changes in connective tissue and collagen. In direct contrast with Fishell et al. (1985), Purchas et al. (2002) speculated that lower ages in the faster-growing group of cattle could have been the cause of the increased tenderness found in their study, yet the faster-growing cattle had higher MFI values, suggesting that the greater protein degradation could be the cause of the discrepancies in tenderness.

Thomson et al. (1999) revealed a higher calpastatin: μ calpain ratio in slower-growing, tougher Simmental steers. In this study, Angus and Simmental steers that grew fastest prior to slaughter were more tender, even though they were older than the slower-growing steers. A greater activity of the calpain system in cattle who reached slaughter weight earliest was reported by Muir et al. (2001) in both growth-restricted and non-restricted steers. Non-restricted steers were heavier at the start of the study, reached slaughter weight faster, and had lower shear force values. Steers slaughtered after the feed restriction and prior to compensatory growth had greater shear force values, higher calpastatin activity, and less m-calpain activity than non-restricted cattle; also, while compensatory growth resulted in fast growth rates, it also resulted in decreased calpastatin, μ-calpain and m-calpain activity were also reduced. These results led the authors to speculate that reduced myofibrillar turnover, potentially caused by nutrition, is linked to decreased tenderization. However, it was noted the restricted steers were older

than the non-restricted steers, and this could potentially account for increased shear force values in the feed-restricted group. It has previously been reported that nutritional restriction resulted in decreased muscle protein synthesis and degradation (Jones et al., 1990), although that study reported an increase in synthesis and degradation after the restriction period was over. Muir et al. (2001), in contrast to what was reported by Jones et al. (1990), proposed that turnover did not increase during compensatory growth, although these authors did not measure protein synthesis and degradation. In contrast to Thompson et al. (1999) and Muir et al. (2001), Therkildsenet al. (2002) did not find a difference in the activities of calpastatin and μ-calpain, or in the calpastatin:μ-calpain ratio between heifers grown at moderate and high rates. High growth rate heifers were found to have significantly greater MFI, significantly lower shear force, and increased sensory tenderness when compared to the moderate growth rate group. The authors attributed this to increased proteolysis based on the high MFI results, although there was no evidence of increased desmin degradation in the high growth rate heifers. However, the lack of difference in calpastatin and μ-calpain activities, along with no evidence of increased desmin degradation, does not support the authors' claims that there is indeed a difference in proteolysis between growth rates.

Consumer Acceptability

It is difficult to set accurate threshold tenderness values for consumer acceptance due to a large variance in consumer preferences (Shackelford et al., 2001). Numerous attempts, however, have been made to quantify tenderness. In the United States,

Shackelford et al. (1991) determined the threshold for slightly tender beef in the retail area was 4.6 kg shear force. Miller et al. (2001) agreed, finding that consumers delineated between tough versus tender at what was determined to be 4.6 kg shear force, with a range between 4.3 kg and 4.9 kg. Feuz et al. (2004), however, reached a slightly different conclusion, and described any steaks with a Warner-Bratzler shear force over 4.0 kg as tough, and any meat with a shear force below 3 kg as tender. In Italy, DeStefanis et al. (2008) reported that consumers viewed steaks with a shear force above 52.7 N (5.7 kg) as tough, while meat with a shear force below 42.9 N (4.4 kg) as tender. Some countries even have threshold requirements for tenderness for quality marketing standards. According to the New Zealand Beef and Lamb Quality Mark Standards, in New Zealand, the average shear force, obtained by using a MIRINZ Tenderometer, must be at or below 8 kg shear force within 24 hours postmortem, and 95% of all samples tested at retail must be below 11 kg shear force (Frazer1997; as cited by Bickerstaffe et al., 2001).

Conclusion

Based on challenges in methodologies used in determining tenderness, and guaranteeing level of tenderness to consumers, the variation in tenderness among steaks is large and has a great deal of economic importance to the cattle industry. As reviewed in detail, tenderness is influenced by numerous pre- and post-mortem factors, but numerous years of research have not led to a conclusion about the effect of growth rate on tenderness. This is in part due to the fact that tenderization is a complex process, and there are still numerous factors not fully understood. New research on tenderness

continually attempts to elucidate relationships between environmental and chemical factors and postmortem protein breakdown, with varying success. Diverse results from studies investigating the relationship between growth rate and tenderness suggest there is indeed an association between how fast an animal grows, the characteristics of its muscle, and how that muscle breaks down during postmortem aging.

Materials and Methods

Steer Selection and Management

One hundred thirty-two sire-verified Simmental × Angus steers born in March and April 2010 at the Bair Ranch in Martinsdale, MT, were shipped to Chappell Feedlot in Chappell, NE in November 2010. Steers were part of the American Simmental Association Carcass Merit Program, through which carcass data is collected from the progeny of registered bulls to aid in the calculation of EPDs. The Simmental Association shared bull data and the Bair Ranch shared identification and early weight data on the steers to allow for steer selection. Ten fast- and ten slow-growing steers were selected from this base population based upon the weights of the steers as determined at four different times during their growth phase prior to harvest. Throughout the study, animals were handled following normal industry practices.

Preliminary selection of steers, used as a guide for animal selection, was based upon birth weight, adjusted 205-day weaning weight, and weight at feedlot entry. Weights were measured and recorded by ranch and feedlot personnel. Weights from each weigh date were ranked from highest to lowest.

While still on feed in February 2011, steers were weighed and ultrasound was used to predict harvest dates, yielding the fourth and final set of weight data. Weight at ultrasound of the twenty preliminary steers was analyzed to ascertain whether or not the steers had maintained their ranking throughout their time on feed. The slow-growing group of steers did not change in the time spent in the feedlot. However, some of the steers that had been preliminarily classified as fast-growing did not maintain their accelerated rate of growth.

Final steer selection was based upon the 205-day weaning weight, weight at entry into the feedlot, and weight at time of ultrasound. The ten steers that were ranked highest in multiple weight times were selected as the fast-growing cattle, and the ten steers that were ranked lowest in multiple weight times were selected as the slow-growing cattle.

One steer from each group (n=2) was harvested prematurely, one due to sickness and one due to feedlot error, resulting in a final number of eighteen steers for the research project. Due to the intensive selection process, no replacement steers were utilized.

Sire information for each of the twenty steers was obtained. Each sire had a negative EPD for shear force indicating that the expectation was that the shear value would decrease compared to the average for the Simmental breed when the specific sire was used. One of the sires had a shear force EPD near zero and the steer sired by this bull was replaced by another steer sired by a bull with a more negative shear force EPD. Attempts were made to balance the sires between groups, with four of the nine bulls having offspring in both the fast- and slow-growing categories (n=12).

All steers received the same feed, environment, and handling throughout their lifespan in an attempt to eliminate confounding variables. After a thirty-day step-up period at Chappell Feedlot, the steers were fed a 94% concentrate feed consisting of rolled corn, beet pulp, dried distillers grains, protein supplement, corn silage, and ground hay, dry matter basis: 14.58% CP and 0.61 Mcal/lb net energy for gain. Steers were implanted in February 2011 with Component TE-S, which contains 120 mg of trenbolone acetate and 24 mg of estradiol USP, along with 29 mg of tylosin tartrate.

Steers were fed at Chappell Feedlot for six months until the slow-growing steers averaged Choice quality grade, as predicted via ultrasound. Steers were then trucked to Columbus, MT and harvested after a 24-hour recovery period following normal industry practices for a small packing plant.

Carcass Data and Sample Collection

Carcass data (hot carcass weight, fat thickness, LD, or ribeye area, maturity score, and marbling score) were collected by an experienced evaluator after a 24-hour chill period. The striploin (NAMP 180) was removed from the left side of the carcass and sliced into 2.54-cm-thick steaks. Steaks were vacuum packaged and aged at 4ºC for 1, 3, 7, 14, or 21 days, then frozen until thawed for analysis.

Shear Force

Shear force measurements were conducted following the procedures of Boles et al. (2009). Briefly, one steak per steer per aging period was thawed at 4˚C for 24 hours prior to cooking. Steaks were broiled in a conventional oven 10.16 cm below the heat

source until the internal temperature, obtained via two Omega Engineering (OMEGA Engineering Inc., Stamford, CT) internal copper-constantan thermocouples inserted near the geometric center of the steak, averaged 70 ± 2˚C. Steaks were turned once when the average temperature was 35º C. After cooking, steaks were placed in a 4˚C cooler for approximately one hour until the steaks cooled. Steaks were weighed pre- and post-cooking to determine cook loss [(raw weight (g)-cooked weight (g)) /cooked weight (g) × 100]. A minimum of five square cores (1.27× 1.27 ×2.54 cm) were removed parallel to the fiber direction from each cooked and cooled steak. Each sample was sheared once perpendicular to the fiber direction with a TMS 30 Food Texturometer (Food Technology Corporation, Sterling, VA) fitted with a Warner-Bratzler shear attachment using a crosshead speed of 100 mm/min to obtain the N required to shear through each sample. The average of the samples sheared per steer was used for statistical analysis. Connective tissue shear force was obtained by measuring the connective tissue peak on the shear deformation curve as described by Moller (1980).

Myofibril Fragmentation Index (MFI)

The myofibril fragmentation of each muscle sampled was measured following the procedures of Culler et al. (1978) as modified by Hopkins et al. (2004). Briefly, 2 grams of muscle per steer per aging period, in duplicate, were minced and homogenized at 15,000 rpm using a Polytron (Brinkmann Instruments, Westbury, NY) for 30 seconds in 10 volumes [wt/vol] of standard salt solution (SSS) [100 mMpotassium chloride, 20 mMpotassium phosphate, 1mM ethylene glycol tetraacetic acid (EGTA), 1mM magnesium chloride, and 1mM sodium azide]. Samples were centrifuged at 4˚C for 15

minutes at 1000 × *g*, the supernatant was discarded, the sample was then re-suspended in 2.5 volumes [wt/vol] of SSS, and then passed through a strainer to remove connective tissue. The sample was then centrifuged, the supernatant discarded, and then re-suspended in 2.5 volumes [wt/vol] of SSS three more times. After the fourth time through the centrifuge, the protein concentration of each sample was determined in duplicate using the Biuret method (Robson et al., 1968). Once the protein content of each sample was determined, samples were diluted with SSS to 0.5mg/ml, and, in duplicate, the absorption of each sample was measured at 540 nm in a Beckman DU 530 spectrophotometer (Beckman Coulter Inc., Brea, CA). The absorption was multiplied by 200 to give the myofibril fragmentation index for each duplicate sample. The use of two samples per steer, with a duplicate taken per sample, yielded 4 measurements per steer. The average of the MFI for each sample (n=2) was calculated, and then the average of the two samples was calculated to give one measurement per steer per aging period. This measurement was used for statistical analysis.

Myofibril Isolation

Myofibril isolation was performed following the procedures of Boles et al. (1992), modified from Lusby et al. (1983).In brief, 2 grams of muscle per steer per aging period were homogenized with a Polytron set at high speed for ten seconds in ten volumes of SSS. The sample was then centrifuged at 1000 × g for 10 minutes at 4˚C. This procedure was repeated with 6 volumes [wt/vol] of standard salt solution and 8 volumes of SSS [wt/vol]. Next, the pellet was suspended and homogenized in 6 volumes [wt/vol] of 1% Triton X-100 made in SSS. The sample was then centrifuged at 1500 ×*g* for 10

minutes at 4˚C. This step was repeated twice before the pellet was suspended in 8 volumes [wt/vol] of SSS and homogenized at setting 3 on the Polytron. The sample was again centrifuged at 1500 × *g* at 4˚C for 10 minutes and the pellet suspended in 8 volumes [wt/vol] of 100 mM potassium chloride before being centrifuged at 1500 × *g* for 10 minutes at 4˚C two times.

Myofibrils were suspended in 10 volumes of 5mM Tris-hydrochloride (Tris-HCL) (pH 8.0) and centrifuged at 3000 ×*g* at 4˚C twice. Samples were then re-suspended in 4 volumes (wt/vol) of 5 mMTris-HCL (pH 8.0) and the protein concentration of each sample was measured using the Biuret procedure modified for use with Tris (Robson et al., 1968). Once the protein concentration was determined, 1 mL of diluted isolated myofibrils were mixed with 0.5ml of bromophenol blue tracking dye [(5% [wt/vol] SSS. 50% [wt/vol] sucrose, 0.05% [wt/vol] bromophenol blue, and 5 mM 2-N-morphinolinoethanesulfonic acid (MES) (pH 6.5)] and 0.1 ml of 2-mercaptoenthenol were added to 1 ml of the sample to yield a protein concentration of 5mg/ml. The samples were then heated at 50˚C for 20 minutes.

SDS-PAGE

SDS-PAGE evaluation of protein degradation was performed following the procedure of Laemmli(1970**)** as modified by Boles et al. (1992). Gels with differing percentages of acrylamide (5% and 10%) and cross-linker (100:1 and 37.5:1) were utilized to evaluate the degradation of myofibrillar proteins. Precast 10% [wt/vol] polyacrylamide Tris-HCl Ready Gels were purchased from BioRad (Bio-Rad Laboratories, Hercules, CA) to use for visualization of proteins with the molecular weight

of approximately 300kDa through 10kDa. Gels contained 12 wells and measured 86 mm × 68 mm. High molecular weight protein degradation was visualized with the use of 160 mm × 180 mm 5% [wt/vol] acrylamide (100 acrylamide: 1N,N' bis-methylene acrylamide) gel, with the final concentration containing 0.1% [wt/vol] SDS [wt/vol], 2 mMethylenediaminetetraaceticacid (EDTA), and 0.375 M Tris-HCL (pH 8.0). No stacking gel was used for the 5% gels.

Gelswere loaded with 10 µl of sample (50 µg of protein) per lane. The PageRuler Plus Prestained Protein Ladder, 10 to 250kDa (Thermo Fisher Scientific Inc, Rockford, IL) containing proteins with molecular weights of 250kDa, 130kDa, 100kDa,70kDa, 55kDa, 35 kDa, 25kDa, 15kDa, and 10kDa was utilized as the standard. Gels were run at 125V on a BioRad Power Pac 300 (Bio-Rad Laboratories, Hercules, CA) until the proteins reached the bottom of the gel, then stained for approximately one hour in 0.1% (wt/vol) Coomassie brilliant blue R-250 (Thermo Fisher Scientific Inc., Rockford, IL), 50% (vol/vol) methanol, and 10% (vol/vol) glacial acetic acid. Gels were then destained in an excess of 50% (vol/vol) methanol and 10% (vol/vol) glacial acetic acid.

Gels were imaged with a Bio-Rad ChemiDoc XRS+ machine with Image Lab software(Bio-Rad Laboratories, Hercules, CA). Band recognition analysis, which functions to visualize dense protein bands that appear in each lane, was used to aid in analyzing the gels.

Statistical Analysis

Data were analyzed using the General Linear Model procedure of SAS. The experimental unit was individual animal. The independent variable was growth rate class,

with shear force, MFI, and carcass data as dependent variables. An analysis of interactions was also performed, between fixed effects of growth rate and day of aging for both shear force and MFI. No significant interactions were found, thus main effects are presented. Correlations between dependent variables were calculated.

After reviewing the raw data it was observed there was a large decrease in shear force value between 1 and 3 days of aging in samples from fast growing steers that was numerically different than values from samples from slow growing steers. In an effort to statistically evaluate this observed difference, analysis of the regression of average daily gain on the slope of the decrease in shear force for days 1 and 3 was performed, but no differences were found. Percentage of Simmental per steer was also inserted into the model as a covariate, but once again, this did not have a significant effect on tenderness. A "by" statement was also inserted into the model, but no statistical difference was found. Therefore, this data will not be presented in the results. Connective tissue force was put into the model as a dependent variable, but as it was not found to have a significant effect on shear force, was not used for the final statistical analysis.

RESULTS AND DISCUSSION

Average Daily Gain

The average daily gain (ADG)of the steers was not used as selection criteria for this study. In hindsight, this would have been the proper way to categorize the steers. This data was not analyzed for statistical differences, but there were numerical differences in the average daily gains between growth rates. The fast-growing cattle had an ADG of 1.33 kg from birth to weaning, and 1.3 kg from weaning until ultrasound. The slow-growing cattle had an ADG from birth to weaning of 0.98 kg, and 1.18 kg from weaning until ultrasound.

Effect of Growth Rate on Carcass Data

Significant differences were observed for two of the carcass characteristics collected by a trained individual twenty four hours after the steers were harvested. In carcasses of fast-growing steers, findings indicated a significant increase in hot carcass weight ($P<0.0001$) and LD area ($P = 0.0093$), while fat thickness, % KPH, and yield grade, marbling, and quality grade did not differ ($P \geq 0.4795$) between growth rate categories (Table 1).

The increase in hot carcass weight and LD, or ribeye, area in animals with an increased growth rateis supported by results from Boles et al. (2009), where cattle sired by Continental bulls had greater carcass weights and LM area than cattle sired by Angus bulls; however, the results differ from those reported by Loken et al. (2009).These

researchers found no difference in these characteristics between Angus-cross steers fed to gain at high and low growth rates. Although the fast- and slow-growing designations in this study were based upon recorded weights of steers that had access to the same feed, numerous other studies investigating the effect of growth rate on tenderness utilized restrictive feeding techniques and reported increased carcass weight in cattle that were not fed a restricted diet (Aberle et al., 1981;Fishell et al., 1987; Calkins et al., 1987; Allingham et al., 1998; Therkildsen et al., 2002). These *ad libitum* groups of cattle may therefore be compared to the fast-growing cattle in the current study, supporting the reported increased in hot carcass weight in fast-growing cattle. It is not surprising that the *ad libitum* cattle had increased hot carcass weights compared to feed-restricted groups in the previously mentioned studies, as the studies were designed to restrict growth in certain groups of cattle.

Table 1.Growth rate main effect least squares means for carcass characteristics

Growth Rate	Hot Carcass Wt, kg	LD Area, cm^2	Fat Thickness, cm	KPH	Yield Grade*	Marbling‡	Quality Grade
Fast	378.38	31.97	0.39	1.94	3.0	401.11	Low Choice
Slow	311.51	29.11	0.39	1.83	2.8	406.67	Low Choice
P-value	< 0.0001	0.0093	0.9337	0.5756	0.4795	0.8628	0.7498
SEM	8.86	0.19	0.03	0.10	0.14	15.82	17.13

*Calculated yield grade = 2.5 + (2.5 × adjusted fat thickness, 12^{th} rib, inches) + (0.0038 × HCW, pounds) + (0.2 × percentage KPH) – (0.32 × LD area, square inches)
‡300 to 399 = slight, 400 to 499 = small, 500 to 599 = modest

Although the majority of the studies listed above did not measure the LD, or ribeye area, an increased ribeye area in the *ad libitum* group of cattle was reported by Aberle et al. (1981) and Fishell et al. (1985). The increased ribeye area implies greater muscling in the *ad libitum* fed cattle, which differed from cattle on restricted diets; Fishell et al. (1985) specifically fed a diet to reduce protein deposition in one group of cattle. The cattle in the current study were not nutritionally restricted, so any increases in hot carcass weight and increased muscling as implied by larger LD area, may potentially be related to the difference in growth rates. It is important to note, however, that the majority of cattle in the fast-growing group in this study were 50% Simmental as compared to 25% Simmental in the slower growing group, which could certainly have influenced the hot carcass weights and LD area sizes, as larger-framed, later-maturing cattle have been reported to produce carcasses with larger hot carcass weights and ribeye areas (Camfield et al., 1999).

In contrast to the results of the current study are the reports of an increase in internal fat (Fishell et al., 1985; Therkildsen et al., 2002), increased fat thickness (Fishell et al., 1985), greater yield grade (Fishell et al., 1985; Calkins et al., 1987), increased marbling (Fishell et al., 1985) and greater quality grades (Fishell et al., 1985; Calkins, et al., 1987)in cattle fed for maximum growth rates. The differences in fat deposition between this study and other reported studies are not surprising. Data reported herein were from steers fed to a specific common marbling endpoint, while the other studies fed the animals to a common weight or to a specific day on feed. There will inherently be more variation in marbling among studies where cattle are not fed to a common marbling

endpoint. Although it is possible to have differences in fat thickness and internal fat in cattle with the same marbling, large differences were not expected, especially in cattle with similar genetics and handling, so it was not surprising that no significant differences were found in any of the carcass characteristics related to fat deposition. The lack of significant differences in yield and quality grades in this study may be explained by the same reasoning.

Effect of Growth Rate on Shear Force

Shear force values were not significantly affected by growth rate (P= 0.3184; Table 2).These results are in agreement with studies by Allingham et al., (1998), Loken et al. (2009) and Calkins et al. (1987). In contrast, Boles et al. (2009) reported an increase in shear force in steaks from animals with larger carcass weights and ribeye areas. In an extreme case of muscling, callipyge, increased muscle growth was associated with increased calpastatin activity (Duckett et al., 2000). As calpastatin is the inhibitor of calpain, this would suggest a decrease in tenderness or increase in shear force with larger carcass weights and ribeye areas. However, in the current study, increased muscling was associated with a faster growth rate, as supported by increased carcass weight and increased ribeye area at a common marbling endpoint but no significant difference in shear force values were found. Following the aforementioned trend, an increased shear force would be expected to be associated with increased muscling, but the findings did not support this premise. Our data also does not statistically support the precedent of increased tenderness in fast-growing cattle and cattle fed for maximum gain (Aberle et

al., 1981; Hansen et al., 2006; Muir et al., 2001; Perry and Thomson, 2005; Purchas et al., 2002; Therkildsen et al., 2002; Thomson et al., 1999).

Any of the differences among the results between studies may be due to the fact that each study utilized animals with different breed compositions, different genetic backgrounds, and experimental designs. Numerous studies reported faster growing animals yielding steaks with greater tenderness than steaks from animals that grew slower; those results were expected from this study. Although this was not the case, the results also did not suggest that faster-growing cattle had tougher meat. As the steers in this study were all fed the same diets, were in the same pen at the feedlot, and were handled by the same staff, these factors are not expected to be confounding variables that would have influenced the results of the study. There are three factors that could have potentially influenced shear force values: the percentage of Simmental in the cattle, the variation in shear force values, both within and among the carcasses, and warmer than expected cooler temperatures at the processing plant where the cattle were processed. As previously mentioned, more of faster-growing cattle had a greater percentage of Simmental compared to the slower-growing cattle. As Continental cattle have been reported to be less tender than British cattle (Boles et al., 2009), and specifically, Simmental cattle have been reported to be less tender than Angus cattle (Chambaz et al., 2003), the greater percentage of Simmental in the faster-growing cattle could have masked any differences between the growth rates.

There was also a large amount of variation among the shear force values, both within individual steers and among the groups. This variation could lead to a lack of

significant difference between growth rates, especially considering the small sample size of this study. Finally, due to the appearance of heat ring on three carcasses and the observation of warm temperatures within the coolers, there is a possibility that the coolers at the processing facility were not as cool as they should have been. This could cause numerous issues in protein degradation postmortem and potentially mask or destroy any apparent shear force value effects. Unfortunately, the thermocouple data to quantify this supposition was lost, and therefore, we cannot specifically determine what the temperatures of the carcasses were.

Table 2. Growth rate main effect least squares means for shear force and MFI values

Growth Rate	Shear Force, N*	MFI
Fast	74.56	57.95
Slow	77.81	58.15
P -value	0.32	0.93
SEM	2.28	1.61

*1 N = 9.81 kg

Effect of Growth Rate on MFI Values

The MFI values also were not significantly affected by growth rate (*P*=0.9289, Table 2). No increase in MFI values in faster-growing cattle disagrees with reports by Therkildsen et al. (2002),Aberle et al. (1981), and Purchas et al. (2002), where the authors concluded that a faster growth rate yielded muscle with increased myofibril fragmentation. However, Fishell et al. (1985) reported a significant effect of growth rates

on tenderness as measured by MFI values and sensory tenderness, but not when shear force was used to measure tenderness. The results from Therkildsen et al. (2002) and Purchas et al. (2002) are to be expected as MFI values have been negatively correlated to shear force values, and those studies reported decreased shear force values in faster growing cattle.

Potential explanations for these results include the percentage of Simmental in the cattle within the groups, and warmer than expected coolers temperatures at the processing plant where the cattle were harvested. Chambaz et al. (2003) reported decreased MFI values at 2 days of aging in Simmental cattle as compared to Angus cattle, although this difference disappeared by 14 days of aging. The greater percentage of Simmental in the steers in the faster growth rate group could therefore have caused a decrease in the MFI values. Something that perhaps had a greater effect on the MFI values in this study is the warm cooler temperatures discussed in the previous section; warm carcass temperatures could certainly cause a more rapid pH decline and an increase in postmortem protein degradation especially in proteins degraded early postmortem. This could have exhausted the protease systems within the muscles, leading to abnormal degradation, and therefore abnormal MFI values, throughout experimental postmortem aging. However, the gels that will be discussed later look very similar to ones published in the literature for similar days of aging, so the decision was made to proceed with the study. Once again, differences in the cattle used in the studies, as well as differences in experimental design and laboratory procedures could explain some of the differences in the results between the previously published studies and the current study.

Postmortem Aging Effects on Shear Force Values

As expected, shear force values decreased significantly(P = 0.0003) as length of postmortem aging increased (Table 3). Numerous researchers have reported a decrease in shear force as days of postmortem aging increased (Monson et al., 2004; Stolowski et al., 2006). This decrease in shear force has been related to increased protein degradation by numerous researchers(Koohmaraie, 1996; Olson et al., 1976; Young et al., 1980). A statistical analysis of the growth rate × day interaction was performed and was not significant for shear force values (P = 0.7809; Table 3).

However, evaluation of raw data suggested that steaks from fast-growing animals had a more rapid drop in peak shear force value from day 1 to day 3 than steaks from slower-growing animals. Shear force values became similar after 7 days of aging, and shear force values for steaks from fast-growing animals continued to decrease up to 21 days of aging, while steaks from slow-growing animals seemed to reach their maximum tenderness after 7 days with little change thereafter. This trend differs from a study by Hopkins and co-workers where lamb chops from slower-growing, feed-restricted lambs had higher shear force values than chops from their fast-growing counterparts (Hopkins et al. 2005). It is interesting to note that in both the current study and the study by Hopkins et al. (2005) the less tender samples appeared to tenderize faster than the more tender samples. However, animals with growth rates that yielded less tender samples differed between studies; it was the slow-growing lambs that yielded less tender meat with a faster drop in tenderization, and in our study it was fast-growing steers that yielded less tender meat initially with a faster drop in tenderization early postmortem.

Huff-Lonergan et al. (1996) reported something similar in shear force values between tough and tender beef samples, but attributed this phenomenon to the supposition that the more tender samples had undergone a period of early tenderization prior to the first sampling period at 24 hours postmortem. However, with the data reported here another plausible interpretation of the information could be postulated: potentially there is a mechanism in these less tender samples from faster-growing cattle that allows them to tenderize faster than the more tender samples or samples from slower-growing animals.

Table 3.Growth rate and length of postmortem aging main effect least squares means for shear force and the interaction of growth rate and aging

	Shear Force, N*
Days aged post-mortem	
1	92.14[a]
3	77.68[b]
7	73.12[b]
14	69.83[b]
21	68.15[b]
P-value	0.0003
SEM	3.61
Growth rate × day interaction	
Fast	
1 day	93.37
3 day	73.42
7 day	73.43
14 day	68.03
21 day	64.55
Slow	
1 day	90.90
3 day	81.94
7 day	72.81
14 day	71.62
21 day	71.75
P-value	0.7809
SEM	5.11

*1 N = 9.81 kg

[a,b]Means within a column without a common superscript differ ($P < 0.05$).

Postmortem Aging Effects on MFI Values

MFI values, as expected, significantly (P<0.001) increased as aging time increased (Table 4), with a positive correlation of r = 0.64405 (P< 0.0001) between the two variables. It has been reported by numerous researchers that MFI values increase with increased postmortem storage, as demonstrated by the results of this study and also studies dating back to the 1970s (Olson et al., 1976; Olson and Parrish, 1977). As was the case with shear force, although the length of aging did significantly impact the MFI values, there was no significant growth rate × day of aging interaction for the MFI values (P= 0.9307; Table 4). It was expected that MFI values would follow the same trend as the shear force data due to the correlation between the two variables; however, the MFI data does not follow the same trend found in the non-significant shear force growth rate × day interaction. Steaks from faster-growing cattle had a drastic numeric drop in shear force between days 1 and 3, but this change was not observed in the MFI data.

Correlation between MFI and Shear Force

MFI values were negatively correlated (r= -0.59058, P< 0.0001)with shear force values. Olson and Parrish (1977) also reported a negative correlation between these variables, although the correlation was greater in their study, ranging from -0.65 to -0.93. This negative correlation has since been corroborated by numerous studies (Chambaz et al., 2003; Crouse and Koohmaraie, 1990; Culler et al., 1978; Vestergaard et al., 2000). The numerous reports of negative correlations between shear force and MFI values means that as shear force values increase, yielding less tender meat, the MFI values

decrease, and vice versa. More tender meat would have greater MFI values, as the MFI measures the ease of fragmentation of muscle; muscle that fragments more easily in a laboratory setting would be anticipated to fragment more easily during chewing, and therefore, be more tender. As expected, the negative correlation found between the shear force and MFI values shows that our less tender samples as measured by shear force had decreased MFI values as compared to the more tender samples.

Visualization of Protein Degradation

Visualization of protein degradation utilizing SDS-PAGE was not analyzed with statistics; planned comparisons between animals were performed, and the results were analyzed visually, with the aid of the ImagePro software band recognition tool. Determination of tenderness was based upon 24-hour shear force values. As Western blot analysis of proteins bands were not performed, we cannot state with certainty the identity of any band as a certain protein; we may only state the apparent molecular weight as they appear on the gels or base the identification on previously reported information.

Results from the 5% gels, used to visualize degradation of large molecular weight proteins, did not show any differences between isolated myofibrils from carcasses of different growth rate classifications with extreme differences in tenderness (Figure 1). There appears to be only T2, the large molecular breakdown product of titin, on the gels no matter the growth classification. There have been multiple reports of a high molecular weight protein, titin, appearing as an intact band- T1 - early in the aging process, which degrades into a second band, T2 (Fritz et al., 1993; Huff-Lonergan et al., 1996). It has

also been reported that there is an earlier appearance of T2 (Huff-Lonergan et al., 1996) and a disappearance of both T1 (Paterson and Parrish, 1986; Huff-Lonergan et al., 1996) and T2 (Anderson and Parrish, 1989) with more tender samples. This was not apparent in our samples.

Table 4. Growth rate and length of postmortem aging main effect least squares means for MFI values and the interaction of growth rate and aging

	MFI
Days aged post-mortem	
1	41.03[a]
3	50.19[b]
7	62.37[c]
14	67.65[c]
21	69.0[c]
P-value	< 0.0001
SEM	2.55
Growth rate × day interaction	
Fast	
1 day	42.48
3 day	49.86
7 day	62.14
14 day	68.03
21 day	67.22
Slow	
1 day	39.57
3 day	50.51
7 day	62.62
14 day	67.27
21 day	70.78
P-value	0.9307
SEM	3.61

[a,b,c]Means within a column without a common superscript differ ($P < 0.05$).

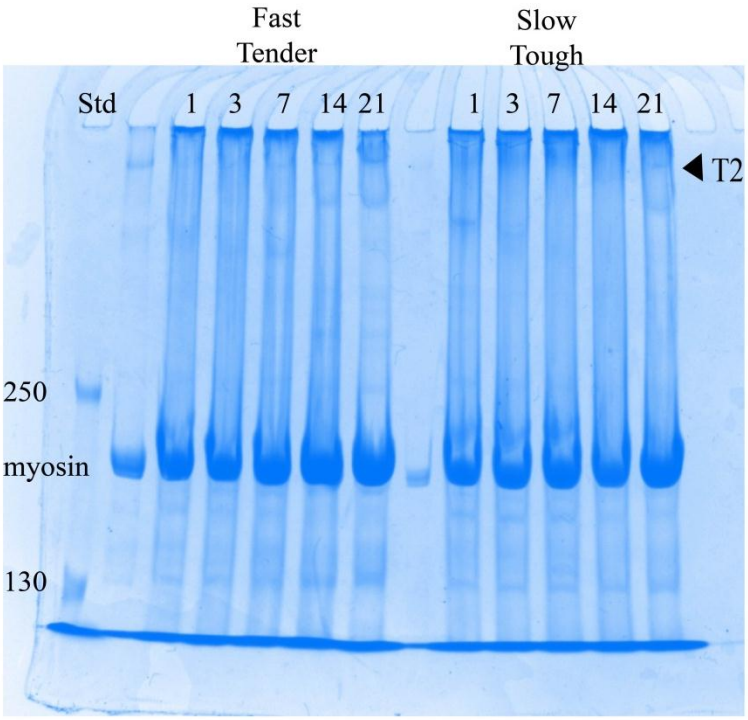


Figure 1. Five percent SDS-PAGE gel of isolated myofibrils from muscle from the most tender fast- and the least tender slow-growing steers from the study animals. Muscle was aged 1, 3, 7, 14, and 21 days. Numbers on the left correspond with the molecular weight standard. Solid triangle indicates band of interest.

A potential cause of no apparent intact titin could be attributed to warmer cooler temperatures during the conversion of muscle to meat. Titin degrades relatively rapidly postmortem and increased carcass temperature could have resulted in breakdown of the protein prior to sampling of the carcass. This was not observed with the lower molecular weight proteins, where samples seemed to separate out into protein bands that are similar to other published results. This is an anomaly that must be acknowledged and is difficult to reconcile. It appears from the gel information that nebulin and intact titin have disappeared by the first sample removed from the carcass at 24 hours, and there is also a

smaller than expected amount of T2. Theoretically the smaller proteins found in the 10% gel should disappear before the large proteins. The fact that this didn't happen either means that the samples just behaved very oddly, or that temperature is not what caused the odd degradation pattern of titin. Large molecular weight proteins are difficult to solubilize during sample preparation (Trinick et al., 1984) and this may have contributed to the anomaly.

Figure 2 compares the differences in low molecular weight protein degradation when comparing the extremes - myofibrils isolated from the most tender of the fast-growing steers compared to the least tender of the slow-growing steers. Two bands of protein appeared different across aging periods. A band with a molecular weight between 130 kDa and 100kDA, appearing below myosin, and one corresponding to the 30,000 dalton component appeared earlier postmortem and with greater apparent density in the most tender, fast-growing animal when compared to the least tender, slow-growing animal. The band near 100kDa that appeared as a triplet after 14 days of aging is potentially an intact band and degradation products or simply multiple degradation products was observed in the more tender, fast-growing animal. This did not occur in the sample from less tender, slow-growing steer.

Figure 3 compares samples from steers of different growth rates with the same shear force after 24 hours. As expected, no differences in the degradation of the 30,000 dalton component were found. There appears to be less degradation of the band near 100 kDa in these samples as compared to the previous gels, but a difference is still seen between the growth rates. While not easily visible on a small screen, there seems to be a

triplet occurring at day 7 in the sample from the faster-growing steer. The sample from the slower-growing steer appears to be a doublet on day 7 that does not appear to undergo further degradation throughout the aging period.

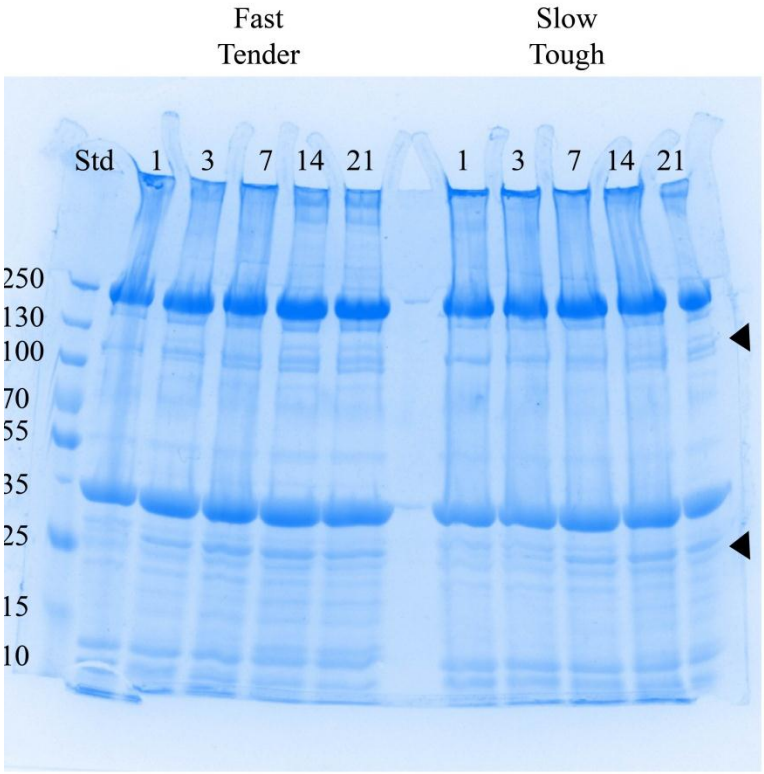


Figure 2. 10 percent SDS-PAGE gel of isolated myofibrils from muscle from the most tender fast- and the least tender slow-growing steers from the study animals. Muscle was aged 1, 3, 7, 14, and 21 days. Numbers on the left correspond with the molecular weight standard. Solid triangles indicate bands of interest.

Figure 4 compares the most and least tender of the fast-growing steers. Once again the protein band near 100 kDa appeared to degrade faster and to a greater extent in the more tender, fast-growing steer when compared to the least tender, fast-growing steer. Band recognition software recognized a doublet of the protein band appearing at day 7 of

aging and a triplet at days 14 and 21 in the sample from the most tender, fast-growing steer. The sample from the least tender, fast-growing steer did not have a doublet appear until day 21, and no triplet ever appeared. There was also an earlier appearance of the 30,000 dalton component in the most tender fast-growing steers: the band recognition software recognized a triplet at day 3 of aging in the more tender steer and at day 7 in the least tender steer.

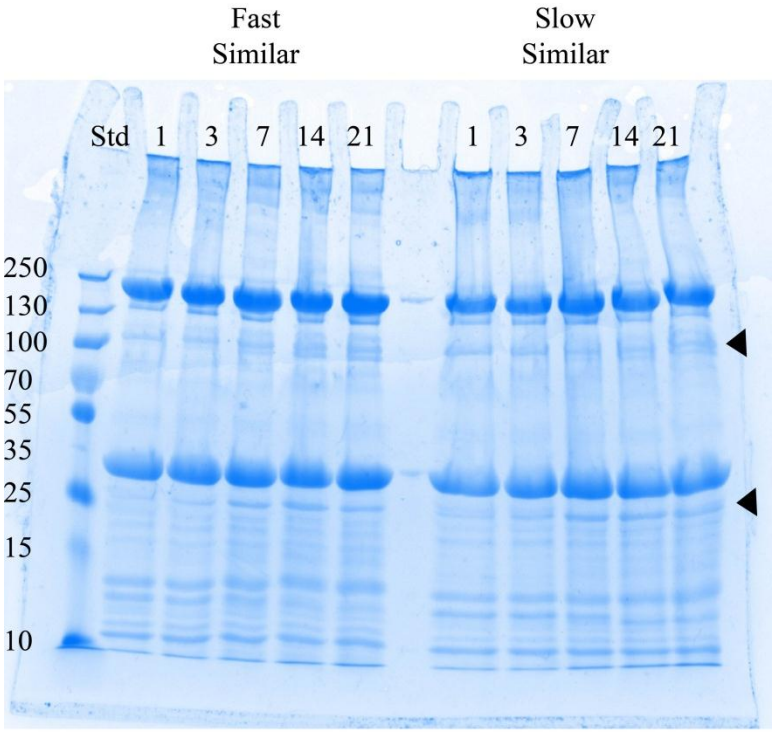


Figure 3. Ten percent SDS-PAGE gel of isolated mofibrils from muscle from steers with the same shear force from different growth rates from the study animals. Muscle was aged 1, 3, 7, 14, and 21 days. Numbers on the left correspond with the molecular weight standard. Solid triangles indicate bands of interest.

Figure 5 compares the most and least tender of the slow-growing steers. The least tender animal is on the left instead of the right as it was in the previous gels. There is no

difference in the degradation pattern of the protein near 100 kDa, either when visualized by the eye or when the bands were analyzed with band recognition software. Overall, the degradation of this protein is not as apparent as it is when comparing the most tender, fast-growing steer versus the least tender, slow-growing steer, and when comparing steers within the fast growth rate. The protein never migrated into a doublet, there was only a single band found through day 21. The 30,000 dalton component does not differ between these two samples even when the band recognition software was utilized; only a single band was present throughout aging.

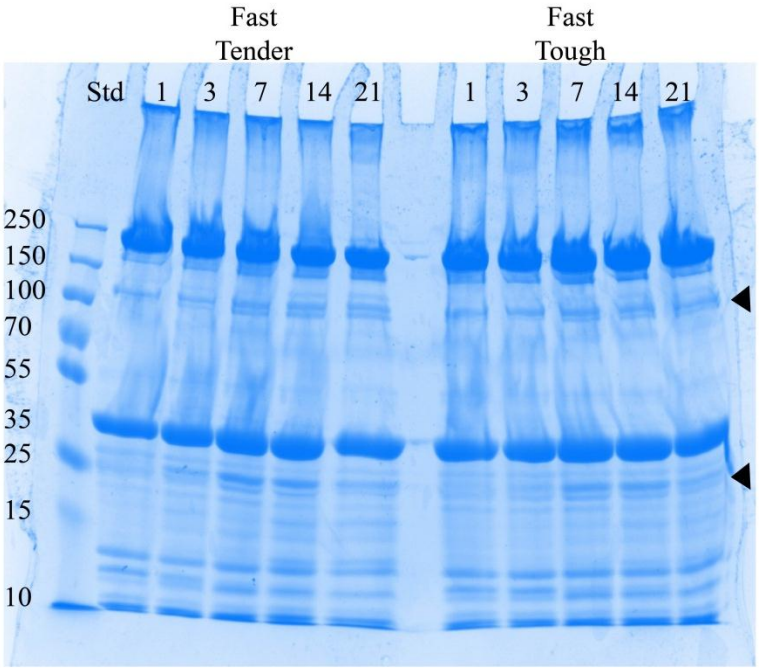


Figure 4. Ten percent SDS-PAGE gel of isolated myofibrils from muscle from the most and least tender fast-growing steers from the study animals. Muscle was aged 1, 3, 7, 14, and 21 days. Numbers on the left correspond with the molecular weight standard. Solid triangles indicate bands of interest.

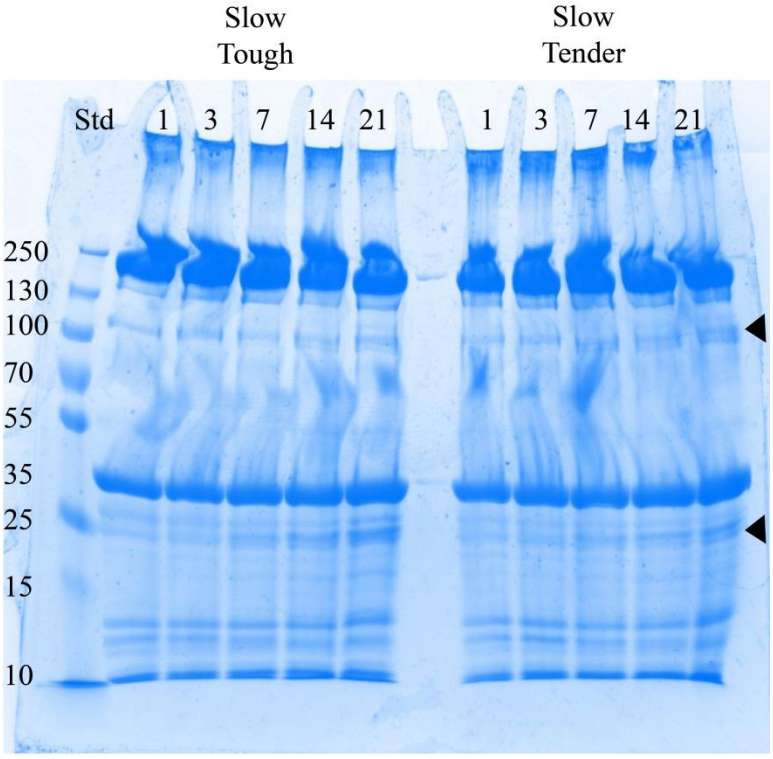


Figure 5. Ten percent SDS-PAGE gel of isolated myofibrils from muscle from the most and least tender slow-growing steers from the study animals. Muscle was aged 1, 3, 7, 14, and 21 days. Numbers on left correspond with the molecular weight standard. Solid triangles indicate bands of interest.

The difference in degradation patterns of the protein band between 130kDa and 100kDa appeared in the gels comparing the most tender, fast-growing steer and least tender, slow-growing steer and the most and least tender fast-growing steers. This protein is currently unidentified in the literature. As the sample from the more tender animal in both of the aforementioned gels (Figure 2 and Figure 3, respectively) showed greater degradation, the first conclusion would be that the band was strictly related to tenderness. However, in the gel comparing degradation between the most and least tender of the slow-growing animals, no differences in the degradation of this protein were observed, and there was a much less dense band indicating less degradation of the protein in

general. The fact that there did not appear to be a difference in degradation between animals in the slow-growing classification indicates that this protein may be related to growth rate and tenderness rather than just tenderness, as the samples came from steers whose meat differed in tenderness. This is corroborated by the appearance of a difference in degradation in the sample from the faster-growing steer when comparing samples from carcasses with the same shear force. As the animals did not differ in tenderness, but did differ in growth rate, it is plausible that growth rate of the steers and not solely tenderness causing a difference in degradation of the protein.

This protein is relatively high in molecular weight, and is contained within muscle at a relatively high concentration based on the intensity of the band on gels. It is possible that the bands are a degradation product of one of the large muscle proteins, and that it has not previously been reported because growth rates and experimental designs between studies may almost never be completely replicated. Although it is possible that warmer than normal temperatures during the first 24-hour hanging period at the harvest facility made the degradation of this protein more visible in the gels, the degradation patterns of the 30,000 dalton component do not differ from those previously reported in literature (Olson and Parrish, 1977; Huff-Lonergan et al., 1996), suggesting that a difference in the degradation patterns of muscle proteins due solely to temperature is not likely.

The earlier appearance and greater intensity of the 30,000 dalton component in the most tender, fast-growing steer when compared to the least tender, slow-growing steer corroborated prior reports of an earlier appearance and greater intensity of this band in more tender meat (MacBride and Parrish, 1977; Olson and Parrish, 1977; Parrish et al.,

1981; Paterson and Parrish, 1986; Huff-Lonergan et al., 1996; Geesink et al., 2001). As expected, there was no difference in the degradation pattern of the 30kDa component on the gel comparing samples from muscles that did not differ in shear force from steers with different growth rates. Gels comparing the most and least tender steers within each growth rate yielded difference in the degradation pattern between growth rates. The most tender, fast-growing steer had an earlier appearance and greater intensity of the 30,000 dalton component as compared to the least tender, fast-growing steer, while there was no difference in the degradation pattern between the most and least tender slow-growing steers. It should also be noted that samples from the slow-growing cattle did not degrade to the same extent as samples from the fast-growing cattle. As seen in figures 4 and 5,a triplet of breakdown components appeared in samples from the fast-growing cattle, but did not appear in samples from slow-growing cattle. These differences in the degradation of the 30,000 dalton component suggest that the extent of the degradation of the 30,000 dalton component is influenced by growth rate.

Study Limitations

It would be impossible to not acknowledge limitations that could affect the interpretation of the data in this study. One such limitation is the small sample size. Nine steers per growth rate group does not lend much power to the statistical analysis. The selection of animals based upon weights rather than average daily gain is also a limitation. In hindsight, average daily gain would have been the better way to select the study steers, as steers with heavier weights might not have necessarily had a faster rate of

growth. Implantation of steers while at the feedlot poses another problem for the evaluation of tenderness, as implants have been reported to reduce tenderness (Boles et al., 2009). However, as all of the steers were implanted on the same day, it is reasonable to assume their responses to the implants would be similar and tenderness was equally affected across the study. Shear force values in this study were very high, suggesting that if implants did decrease tenderness, it occurred in all steers, but this supposition is not verifiable, as it is impossible to measure tenderness pre- and post- implant in the same animal. Another limitation to the study is the possibility of uneven sire distribution between the groups. Attempts were made to balance the sires between groups, but it was not possible to do that while maintaining the large differences in growth rates that we desired; only four of the nine sires had offspring in both groups.

The fourth limitation to this study is differing percentages of Simmental genetics between groups. The majority of the cattle in the fast growing group were 50% Simmental, while the majority of the cattle in the slow growing group were 25% Simmental. As meat from Continental cattle has been reported to be tougher than that of British breeds (Chambaz et al., 2003; Boles et al., 2009), the greater percentage of Simmental genetics in the faster growing group of steers could have caused the decreased tenderness that we saw. It is also possible that there was not enough heterogeneity within the study animals, and that the population was too homogenous for any differences to appear. There is also the possibility that human error in the laboratory occurred and impacted the study results, as there were multiple people who performed lab work. This

is especially possible in the case of sample preparation for the SDS-PAGE myofibrils, as the high molecular weight proteins did not appear to isolate well.

The final limitation is the aforementioned failure of the coolers at the harvest facility. Due to the possibility of increased protein degradation due to warmer temperatures, we may not state with certainty that differences in protein degradation observed were due to growth rate. However, as there was no difference in fat thickness of the carcasses, it is likely that the cooling of carcasses occurred at relatively the same rate. Although the carcasses of fast-growing steers were significantly heavier than the slow-growing steers, which could cause slower cooling of the carcasses, the results of MFI and shear force evaluations do not support this. MFI values of both growth rates increased over time in a manner similar to results from previous studies (Olson et al., 1976; Olson and Parrish, 1977; Olson et al., 1976). Research on high-temperature conditioning of beef carcasses reported increased MFI values throughout 14 days of aging from samples from carcasses held at a high temperature (Whipple et al., 1990a), and slow chilling of beef carcasses has been reported to result in reduced shear force values that did not decrease to a great extent over time in carcasses (Hwang and Thompson, 2001). There was no difference in the MFI or shear force values between growth rates in the current study, suggesting that the increased muscling in carcasses of fast growing steers did not negatively affect chilling rate.

Summary

Results from this study on the relationship of the growth path of steers to carcass composition and meat quality were inconclusive. Although hot carcass weight and LD area of the carcasses from fast-growing steers were significantly greater than from slow-growing steers, implying greater muscling, there were no statistical differences in the shear force or MFI values. Although there were no differences due to growth rate in the objective measurements of tenderness, visualization of protein degradation with SDS-PAGE gels did suggest that there is a difference in the degradation of an unidentified protein band that is potentially related to growth rate. There was also a numerically greater decrease in the average shear force values between days 1 and 3 of aging in the samples from faster-growing steers. Combining SDS-PAGE information with shear force values begs the question of whether this faster decrease in shear force is related to simply the tenderness (or lack thereof) of the samples, or if it is related specifically to the growth rate of the cattle involved. I would hypothesize that faster-growing cattle do appear to have a mechanism allowing their meat to tenderize faster, but that this mechanism is overshadowed by variation among animals in different studies. This could potentially explain part of the variation in tenderness found in the marketplace, and we feel that more research on the subject is warranted. The unidentified protein must be identified, and its relationship to tenderness must be established. Efforts must then be focused on elucidating the relationship, if any, between growth rate and the degradation of the currently unidentified protein to determine if the growth rate of steers is a factor that may be used to identify tenderness postmortem.

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