EFFECT OF TEMPERAMENT AND GROWTH RATE ON TENDERNESS OF BEEF STEAKS FROM SIMMENTAL CROSS STEERS

by

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ABSTRACT

Tenderness has been identified as one of the most important traits consumers think about when choosing a steak. Unfortunately, the variation and lack of tenderness in steaks has created a negative eating experience for consumers. Recent research has raised the question if temperament as measured by exit velocity and docility scores could be a factor influencing variation in tenderness. However, docility scores are applied subjectively and exit velocity measurements require timers that can be expensive. An objective score that could be used would be helpful. Animals under stress often revert to anaerobic metabolism, especially in the muscle. Anaerobic metabolism results in elevated blood lactate suggesting the use of the small blood lactate meters as objective measures in identifying an animal's response to stress. The objective of this study is to determine if temperament effects growth rate and overall tenderness of beef steaks, and can blood lactate be used as an objective measurement of temperament. One hundred and fifty four steers were evaluated for exit velocity, blood lactate and docility score. Steers were humanely harvested and carcass data obtained. Loins from 30 steers were obtained and cut into steaks. The steaks were aged 3,7,14, and 21 days postmortem and used for shear force, and myofibrillar fragmentation index analysis.

All temperament measurements were significantly correlated meaning if average lactate measurement went up the exit speed was high along with the docility score being higher. Temperament and growth classification significantly affected carcass weight. The animals that left the chute more slowly had lower carcass weights than did the steers that left the chute at a fast or medium rate. Additionally, blood lactate significantly affected shear values. Shear was significantly correlated to growth rate, along with blood lactate level. In conclusion temperament did not affect growth rate but did have an impact on tenderness. Our results suggest that growth rate, exit velocity and blood lactate contribute to variation in tenderness. Finally, if an animal is temperamental based on exit scores and lactate concentrations before harvest this could set the stage for postmortem processes that contribute to the high variance in tenderness in the marketplace.

1. INTRODUCTION

Consumer purchasing decisions are heavily affected by the palatability of beef products, and several factors including tenderness have been shown to affect this (Wulf et al, 1996). Additionally, consumers have consistently ranked tenderness as the most important attribute to eating quality and overall palatability of beef (Peachey et al, 2002, Duizer et al, 1993, Moeller and Courington, 1998). Koohmaraie et al, (2002) stated that solving inconsistent meat tenderness was a top priority to consumers. The National Beef Quality Audit conducted in 1991 showed that the variation in tenderness was of great concern to consumers purchasing a steak (Shackelford et al, 1995). King and coworkers (2006) reported consumers could discriminate between tenderness of steaks and were willing to pay more for "guaranteed" tender beef. Lack of consistent tenderness increases the risk of a poor eating experience by the consumer (Eilers et al, 1996). In turn, this has led the beef industry to attempt to produce a consistent, tender and cost appealing product to consumers everywhere (Behrends et al, 2009). Tatum et al, (1999) suggested a total quality management approach to meat which addressed the incidence of variation in tenderness would result in more consistent and tender product. To achieve the more consistent product it is important that we understand all of the possible ante- and post-mortem factors that impact tenderness.

Increased demand for lean meat products has lead consumers to choose meat with lower visible fat and therefore lower quality grades. This trend has been paralleled by increased incidences of poor eating experiences (Wulf et al, 1996). These poor eating experiences have in turn resulted in a negative perception of the beef industry. (Cross et

al., 1984). Cross and co-workers (1984), along with Monson et al (2004), reported that even though consumers indicated they wanted leaner beef choices they actually were less happy with leaner product because of inconsistent tenderness.

Boleman et al, (1997) found that consumer perceptions of steaks of known shear force and buying trends follow tenderness patterns, where consumers were known to pay a premium for improved tenderness. These researchers found that with the ability of consumers to discriminate between tenderness categories and their stated willingness to pay a premium for tender beef, it would be possible for economic incentives to be used from retailers to packers and on to producers to promote the production, identification and marketing of tender beef.

Consumer acceptance is the most important attribute to product success. According to the 2001 to 2004 Beef Industry Long Range Plan, improving consumer satisfaction with palatability and consistency of beef products was one of the US beef industry's key strategies for attaining the goal of increasing consumer demand for beef by 6% (Platter et al, 2003). Results from the National Beef Tenderness Survey (Morgan et al, 1991) revealed that beef was too variable in tenderness. National Consumer Retail Beef Study used quality grades to categorize steaks to be evaluated by consumers. Various degrees of tenderness within each category were found by consumers. This information that quality grade alone was insufficient to guarantee the tenderness of a steak.

Café et al, (2011) found cattle with more excitable temperaments, measured by flight score and chute speed had less feed intake and slower growth rate than cattle with

less excitable temperaments. Additionally, Voisinet et al, (1997) along with Behrends et al, (2009) found a decrease in growth rate as measured by average daily gain in animals with more excitable temperament. This was related to higher shear force values, along with increased number of dark cutting carcasses (Voisinet et al, 1997; King et al, 2006; Behrends et al, 2009). This led producers to question production practices that may lead to increased stress and also to consider selection of animals based on temperament in order to improve tenderness.

In conclusion, research indicates consumers preferred eating satisfaction is to a certain degree determined by tenderness of a steak. Unfortunately, the industry hasn't yet found a way to produce a consistently tender steak. Additionally, the question of different factors impacting tenderness is important to develop control mechanisms to improve the eating experience of the consumer.

2. LITERATURE REVIEW

Muscle Ultrastructure

Meat is defined as edible skeletal muscle from livestock. Meat can be divided into several categories including red meat, which has been traditionally defined as beef, pork, lamb, mutton or veal. (Aberle, Forrest, Gerrard, and Mills, 2012). The structure of the muscle is important to the tenderness and functionality of meat as well as being important to the understanding of results obtained in research.

Muscle fibers make up a large proportion of skeletal muscle. These fibers are comprised of proteins that are grouped into three categories based on the solubility of the muscle proteins. Salt soluble proteins are the proteins most often associated with contraction including myosin and actin. These proteins are soluble in salt solutions with an ionic strength greater than 0.18. The proteins soluble in water or low ionic strength solutions are mostly enzymes but also contain the meat pigment myoglobin and are categorized as water soluble. The last category are those proteins that are not salt soluble and are made up of mostly connective tissue proteins, but also include titin and nebulin, two cytoskeletal proteins. Additionally, myofibrils are made up of repeating units called sarcomeres which when contracted slide filaments past each other to shorten the muscle. The proteins that make up the sarcomere and the three dimensional structure are responsible for the texture of meat. (Aberle et al, 2012).

Sarcomere

The sarcomere has a light and dark banding pattern when observed under polarized light. The Z-discs or Z-line represents the lateral boundaries of the sarcomere. The light band, also called the I band is isotropic under polarized light and contains the thin filaments. The dark band in the center of the sarcomere, the A- band, contains mostly the thick filaments and some overlapping of the thin filaments. In the center of the A-band there is a lighter zone known as the H-zone that is bisected by the M-line. The H-zone is the distance between thin filaments extending into the A band. A third filament system, also known as gap filaments (Clark et al., 2002) are made up of a single molecule of titin, which spans the distance from the Z line to the center of the sarcomere anchoring to the M-line. Nebulin, creates another filament system that runs parallel to the thin filaments and is thought to determine the length of the thin filament during fiber development (Clark et al., 2002).

Z-Line

The Z-line defines the lateral boundaries of the sarcomere, along with establishing an anchoring site for the thin, titin, and nebulin filaments. Due to this anchoring property, Z-lines are the primary channels of force generated by contraction. Yamaguchi et al, (1985) reported that all vertebrate Z-line structures have a common, basic structural unit. The co-existence of these structures within a limited area of a single section, suggests that there must be a dynamic relationship between these structures and the Z-filament positions. Furthermore, the width of the Z-line is determined by the amount of overlap of thin filaments from adjacent sarcomeres, and the associated increase in number of Z-line

units (Yamaguchi et al, 1985). Golstein et al, (1986) discovered changes in the Z-line ultrastructure (structure within a cell) with contraction. These researchers found small square patterns in Z-bands from entetanized control rats, a small square pattern predominated (>80%) in Z-bands from muscle from tetanized-rest control rats and a basket-weave pattern predominated (>90%) in soleus muscle Z-bands fixed during tetanic contraction, indicating a change in the spacing of Z band proteins in response to contraction. Golstein et al, (1986) predicted that the transition from one lattice to another might occur at any point in the lattice where the axial spacing changed. These researchers explained this transition by a shift in the longitudinal spacing of the cross-connecting Z-filament attachment points.

The Z-lines of adjacent myofibrils are aligned providing a means to coordinate contractions between individual myofibrils to a point where the Z-line is linked to muscle membrane. Additionally, the Z-line has a role as a biomechanical sensor that can respond to changes in tension at the sarcolemma (sheath that envelops the fibers of skeletal muscle). Therefore, the Z-line is not just the border, but plays an important role in signaling and muscle homeostasis (Golstein et al, 1986).

A-Band

The A-Band is located between the end of one thin filament, through the M-line, to the opposite thin filament. Luther and Squire, (1978) stated that myosin filaments that form the A-bands in vertebrate skeletal muscle are cross-linked halfway along their length by a structure called the M-band. Additionally, Woodhead and Lowey, (1983) reported the M-band appeared in electron micrographs of longitudinal sections of many

types of striated muscle as an electron-dense region traversing the center of the A-band. The M-Line is the main anchoring site for thick filaments, and is considered to be the final step in myofibril assembly as defined by restriction of thin filament length and the clear formation of two half sarcomeres (Clark et al, 2002). The H-Zone is a lighter area found within the A-band, between the ends of the thin filaments (Greaser, 1991). Additionally, the pseudo H-zone of the sarcomere is located along each side of the M-line, where thick filaments lack myosin heads (Sawada et al, 1978).

Thick Filaments

In the myofilaments, the longitudinal section, also known as the thick filament is aligned parallel to each other and arranged in exact alignment across the entire myofibril. Furthermore, thick filaments of vertebrate muscles are approximately 14 to 16 nm, constituting the A band of the sarcomere (Aberle et al, 2012).Thick filaments are made of myosin and associated proteins (myomesin, M-line protein and C-protein). The pointed ends of the thin filaments interdigitate with the thick filaments within the A-band. Globular heads of the myosin molecules, known as cross-bridges extend from the core of the thick filaments and cyclically interact with the thin filaments (Clark et al, 2002).

Thin Filaments

Thin filaments are about 6 to 8 nm in diameter and extend on each side of the Z disk. These constitute the I band of the sarcomere and extend beyond the I band into the A band (Aberle et al, 2012). Thin filaments, also known as the actin filament, are anchored in the Z-disc, span the I-band, and extend toward the M-line. The thin filament

backbone is made up of actin. Actin monomers join together to form two twisted alpha-helices to form the core of the thin filaments. The thin filaments are capped at the pointed and barbed ends by tropomodulin and CapZ (Clark et al, 2002). Furthermore, each strand of tropomyosin lies within each groove of the actin super helix (Aberle et al, 2012).

Myosin

Clark et al, (2002) stated thick filaments are composed of hundreds of myosin molecules. The myosin molecule contains two heavy chains (MHC) and four light chains (MLC), where two of the light chains belong to the essential light chain (ELC) family, and the other two are regulatory light chains (RLC), functioning mainly to make adjustments to myosin motor activity (Milligan, 1996). The myosin molecule is characterized into two functional regions, the head and the rod. The head domain is made up of the N-terminal region of each MHC and two light chains to make up the myosin head domain that forms the actomyosin cross bridges. The head domain, forms a catalytic motor domain, and contains the binding sites for actin and nucleotides. During contraction, the hydrolysis of each ATP molecule causes a large rotation of the head domain to interact with actin, resulting in the powerstroke, where ADP is dissociated and the actomyosin complex returns to the relaxed state (Clark et al, 2002).

Actin

Actin exists as two different forms, g- actin or globular actin and f-actin or filamentous actin. The f-actin comprises the backbone of the thin filament. Actin monomers (g-actin) join together to form f-actin and develop into two twisted alpha-

helices to form the core of the thin filaments. Each half-of a helical turn of the thin filament is comprised of 7 actin monomers. The length of the f-actin is determined by nebulin. The motor activity of the myosin heads attached to actin moves the thin filaments past the thick filaments, generating force and resulting in muscle contraction (Clark et al, 2002).

Tropomyosin

The primary function of tropomyosin is to work together with troponin to regulate the interaction of the thin and thick filaments (Clark et al, 2002). Tropomyosin is constructed of two alpha-helical chains arranged as a coiled-coil rod, which lies along the groove of the actin filaments, covering the myosin binding site on each actin monomer. In striated muscle, all tropomyosin span 7 actin monomers. The binding of tropomyosin along each of the two grooves of the actin filament is highly cooperative and occurs in a head-to-tail manner, overlapping approximately 8 to 11 amino acids (Wegner, 1979). Tropomyosin also stabilizes the thin filaments by slowing depolymerization of the actin monomers at their pointed ends, where it interacts with the capping protein, tropomodulin. The control of myosin interaction with actin during contraction by tropomyosin involves Ca^{2+} and the troponin complex. Calcium binds to the troponin complex causing a conformational shift in the position of tropomyosin on the thin filaments. Upon the calcium binding tropomyosin shifts its position to expose the weak myosin-binding sites on actin allowing interaction between the two filaments and thus slide the Z-lines closer together. (Clark et al, 2002).

Troponin Complex

Troponin is made up of three different subunits, troponin I, troponin T and troponin C. Troponin I is the inhibitory subunit of the troponin complex and it completely inhibits actomyosin ATPase activity in vitro (Westfall and Metzger, 2001). The Ca^{2+} dependent regulation of contraction in vivo requires the presence of troponin T, C and tropomyosin. In the off-state, the N-terminal end of troponin I is bound to troponin C and troponin T, and the C-terminal region is tightly bound to actin. Upon Ca^{2+} binding to troponin C, the affinity of troponin I for actin is reduced whereas its affinity for troponin C and troponin T is enhanced. This change in affinity allows for the movement of troponin and tropomyosin away from the myosin binding site of actin. Troponin T is thought to anchor troponin C and troponin I dimers in the thin filament, functioning as the molecular organizer of the thin filament regulatory complex. (Clark et al, 2002).

Titin

After actin and myosin, titin is the third most abundant muscle protein and is the largest single subunit protein identified. Titin makes up a third filament system (Wang et al, 1979, Maruyama et al, 1977) that appears to be a molecular spring that governs the stiffness of the sarcomere. Clark et al (2002). Additionally, Clark et al, (2002) stated titin, also known as connectin, connects the Z-line to the M-line in the sarcomere. The N-terminal ends of titin, from adjacent sarcomeres overlap in the Z-line and span the I- and A-bands, with the C-terminal ends overlapping in the M-line, thus forming a continuous filament system in myofibrils. (Clark et al, 2002).

Nebulin

Another component of the sarcomere is nebulin, a very large actin-binding protein, located parallel to the thin filament of the sarcomere and is known to regulate actin-myosin interactions by inhibiting ATPase activity in a calcium-calmodulin sensitive manner (Clark et al, 2002). Nebulin is a giant protein that forms the fourth filament system in skeletal muscles. The C-terminal end of nebulin is partially inserted into the Z-lines, with the N-terminal end extending to the pointed ends of the thin filaments (McElhinny et al, 2001). Nebulin appears to assemble early during myofibrillogenesis, before actin filaments attain their mature lengths and organization (Ojima et al, 1999). The nebulin filament acts as a molecular ruler for specifying the precise lengths of the thin filaments.

α – Actinin

Alpha – actinin, a major component of the Z-line, contains three major domains, a globular N-terminal actin binding domain, a central rod domain composed of four spectrin-repeats, and a C-terminal domain. The rod domains of α-actinin monomers interact to establish antiparallel dimers that are capable of cross-linking actin and titin filaments from neighboring sarcomeres. Even though α-actinin is a prominent component of the Z-disc, it is not essential for the proper assembly of the thick and thin filaments machinery (Clark et al, 2002).

Desmin

Desmin makes up intermediate filaments and play important roles in the mechanical integrity and elasticity of muscle cells. The most frequently observed feature of desmin is an initial unbinding transition that corresponds to the removal of coiled-coil dimers from the filament surface (Kiss et al, 2006). Desmin surrounds the Z-lines of the myofibrils and connects adjacent myofibrils at the level of the Z-lines (Yagyu et al, 1990). Desmin is degraded early postmortem and its degradation has been related to more tender steaks.

Calpain System

Calpains are a group of calcium activated proteases that are thought to initiate metabolic turnover of myofibrillar proteins, resulting in a major influence on rate of muscle protein degradation (Goll et al., 1992). These proteases were originally called CAF or calcium activated factor (Goll et al, 1992).Two isoforms have been identified in muscle. These two isoforms have different calcium activation requirements, m-calpain (mMol) and μ - calpain (μMol) (Huang and Forsberg, 1998). Calpains are regulated by a variety of factors including calcium and calpastatin (Huang and Forsberg, 1998). Evidence suggests that the calpain system is related to the initiation of muscle turnover (Goll et al., 1992). Researchers have reported complete removal of the Z-line when muscle samples were incubated with calcium and calpain (Goll et al., 1992). Further evidence is that calpains breakdown other proteins that create the scaffolding of the sarcomere including C – protein, troponin-T, tropomyosin, titin, nebulin and desmin.

More evidence that calpains initiate turnover but are not completely responsible is that they have little to no effect on native myosin and actin (Goll et al., 1992).

Calpains have been associated with increased tenderness with postmortem ageing. Goll et al, (1998) indicated that calpains were responsible for up to 95% of all proteolysis and postmortem tenderization that occurs during the first 7 to 14 days of postmortem storage (2°C). Furthermore, during skeletal muscle postmortem ageing, the Z-disk degrades, leading to weakening and fragmentation of myofibrils, along with the degradation of desmin, causing disruption of cross-linking between myofibrils and fragmentation of myofibrils. All of these postmortem changes are seen when myofibrils are incubated with calpain.

SDS-PAGE of myofibrils after incubation with the calpains shows that the calpains have a very limited and specific effect on myofibrillar proteins. Goll et al (1992) reported a very large polypeptide fragment migrating above the myosin heavy chain that appears after 5 or 10 minutes, which is likely to originate from titin because titin is the only protein in myofibrils that is present in sufficient quantities to produce a prominent fragment that is larger than 300 kDa (Goll et al, 1992).

Additonally, Goll et al, (1992) inbdicated μ-calpain and m-calpain were shown to degrade Z-disks, troponin T, and C-protein, so the thick and thin filaments would be released from the sarcomeric structure. Furthermore, researchers found that after incubation with the calpains, the resulting myofibril is narrower by two thick filaments and two thin filaments. Finally, researchers found the thick and thin filaments released by

the calpin may either re-associate with the myofibril or be degraded by cytosolic proteases (Goll et al, 1992).

Proteasomes

Proteasomes are large multisubunit proteases (Bochtler et al., 1999). Proteins must be able to enter into the barrel shape of the proteasome to be broken down. This prevents very large structures from being broken down. The proteasome is activated by the addition of ubiquitin to proteins as a marker and has been shown to degrade purified actin and myosin (Huang and Forsberg, 1998). However, Solomon and Goldberg (1996) reported that ubiquination of myosin was inhibited by the presence of actin and therefore disassembly of the filaments was necessary supporting the assumption that calpains activate the muscle turnover by disassembly of the filaments to allow for the ubiquitin – proteasome pathway to be activated.

Conversion of Muscle to Meat

In conversion of muscle to meat, many factors within the body and outside the body can change the biochemical pathway muscle goes through. Two of the factors that have a great impact on the conversion of muscle to meat is temperature and pH. Temperature is important as it affects the rate of metabolism postmortem. Increased temperature increases the rate of reactions and thus affects the quality of the meat (Aberle et al, 2012). After exsanguination, aerobic metabolism is reduced as oxygen is limited, causing pyruvate to form lactate instead of entering into the citric acid cycle and then continue metabolism in the mitochondria through the electron transport chain. The

conversion of metabolism to anaerobic pathways leads to the buildup of lactic acid in the muscle. Furthermore, anaerobic metabolism produces less ATP leading to the depletion of ATP. During rigor mortis, also known the stiffness of death, ATP is not available to release actin from myosin so permanent cross bridges form. Another factor in this conversion process is the decline of pH due to lactic acid build up. A normal pH decline in muscle is a gradual decrease from approximately 7.4 to 5.6 within several hours, and results in normal color and texture of meat.

Glycogen

Immonen et al, (2000) stated muscle glycogen is the main metabolic substrate responsible for postmortem lactic acid accumulation and normal pH decline. Researchers stated the concentration of glycogen varies greatly at the time of slaughter depending on the muscle, species, and nutritional status of the animal. These researchers identified pre-slaughter stress to have the biggest impact on glycogen stores. Researchers the independent effects of residual glycogen concentration on the physical and sensory quality of normal-pH beef were quite numerous but moderate in size. The formed categories (≤25 Mmol of glycogen/kg, 25.1-49.9 mmol/kg and ≥mmol/kg) differed significantly in residual glycogen concentration ($P<0.0001$) as well as in ultimate pH (48h)($P<0.005$). Furthermore, residual glycogen by itself or in interaction with ageing had various statistically effects on the physical and sensory quality of beef, like juiciness, shear force, and thawing loss. The thawing loss of beef was significantly affected by the residual glycogen concentration. There was no explanation why at thawing, or perhaps at freezing, the water bound in glycogen molecules seemed to become loose. Researchers

concluded that of all the quality effects of residual glycogen concentration were found in the lowest and the highest glycogen category not the intermediate category.

Immonen et al, (2000) stated muscle glycogen concentration at the time of slaughter is one of the most important factors affecting beef quality. Additionally, researchers stated the ultimate meat pH and residual glycogen concentration reflected the glycogen stores created by experimental diets. Limited decline of pH is known to result from low glycogen stores in the live animal (Café et al, 2010) and due to pre-harvest handling, elevated pH in meat can result (Watanabe and Devine, 1996). Various ante-mortem stress factors are responsible for depletion of glycogen or lowered glycogen stores including duration of transportation or lairage, diet, climate, and genetics (Silva et al, 1999). Furthermore, Bowling et al, (1977) found that grass-fed steers had higher ultimate pH values than grain-fed steers and suggested that the grass fed cattle were more susceptible to pre-slaughter stress because of lower glycogen stores, resulting in higher pH values.

Methods of Measuring Tenderness

Texture, paired with tenderness has been determined to be one of the most important factor to palatability and a factor with the most variation (Brady and Hunecke, 1985 and Butler et al., 1996). Texture is the result of structure of food, which is composed of several different properties. Humans are the best instruments to evaluated texture, because humans are the only devices that can interpret and have a different number of textural sensations at the same time (Brady and Hunecke, 1985). Brady and

Hunecke (1985) stated that tenderness is highly related to food texture, which is defined as the composite of those properties, which arise from the structural elements and the manner in which it registers with the physiological senses. Numerous surveys have indicated that tenderness or toughness of beef is the sensory factor that contributes most to eating satisfaction or dissatisfaction. Sensory assessment of meat quality is obtained by use of either trained taste panels or untrained consumer panels (Perry, 2001).

Methods for assessing tenderness can be either subjective or objective. Consumers usually participate in sensory or subjective tests during product development, in order to screen products, and test their acceptance. These are used to determine if a consumer likes or dislikes a product, and are designed to test the market demand of certain products. These tests indicate the acceptance of a consumer without the package, label, price, and any other outside deciding factors. When subjectively analyzing the texture of meat with sensory analysis, tenderness is an attribute that changes over time from the initial bite and continues through mastication, while the teeth and saliva are breaking down the bite. The continued chewing of the product results in a residual bolus right before the bite is swallowed. The amount of residual bolus is not always consistent (Butler et al., 1996).

Two types of sensory panels are commonly used to evaluate food products, acceptance or preference tests (consumer testing) and quantitative scales (trained panels). Preference tests, describe an experience or an attitude towards a product. This is created by ratings of the consumer to measure the acceptability or liking of the food. The paired preference, ranking, and rating tests are the three types of consumer tests. The paired

preference test is when two samples are presented and the panelists are asked to determine which of the two products is preferred. This is beneficial when there are more than two samples, as it is easy to organize, however the presentation of sample must be equal among all consumers in order to maximize accuracy. Conversely, the paired test provides much less information than the ratings test because it gives no measurement of degree of the "likeness" that the consumer has towards the product.

In regards to the consumer panel, sampling is one of the most important components in conducting the panel. It is important to get a random assortment of people from the population. Identifying the target population is important for the study as it is the segment of the population that is known to regularly purchase products like the one you are testing.

Trained sensory evaluation using quantitative scales is a common practice in order to assess the quantity of difference between samples. Three areas must be controlled in order to achieve true repeatability of the data; these include the environment, the selection and training of panelists and the type and structure of each of the tests. It is crucial to control the environment, as it can easily interfere with results. Removing as many confounding factors as possible is important to assure that the responses of each panelist are consistent.

Meat can be evaluated for the amount of juiciness, tenderness, connective tissue amount, flavor intensity, off-flavor characteristic and off-flavor intensity. Aromatics as well as tastes are used to describe the samples as grainy, cardboardy, painty, fishy, livery, soured, and medicinal. Additionally, tastes and feeling factors can be described as

metallic, astringent, throat irritation, and chemical burn as well as salty, sour and bitter. Care must be used when conducting sensory evaluation to make sure the question asked is the question answered.

The objective methods of measuring tenderness range from simple determinations of tenderness or toughness by the Warner-Bratzler shear force to more complex practices trying to measure the pressure exerted by teeth (Bouton et al., 1975, Moeller and Courington, 1998). The most popular way to measure tenderness objectively is the Warner Bratzler Shear device, which is used to evaluate specific changes in myofibrillar and connective tissue components of tenderness in samples of meat (Moller, 1981, Purchas and Aungsupakorn, 1993). The meat sample is placed on the plate, and the V-shaped blade is pushed through the slot, the meat is compressed and finally broken in half. The force that is required to break the piece of meat in half versus the amount of time is recorded. The Warner Bratzler Shear analysis results in a parabola shaped curve, where the peak force is determined as the force in amount of kg or Newtons it takes to shear perpendicularly through a core of meat. The maximum peak is what is known as the peak WB shear force (Zhang and Mittal, 1993). Shear measurement based on the Warner Bratzler shear force have a higher correlation with myofibrillar or muscle fiber properties than with connective tissue properties (Graafhuis et al., 1991). Graafhuis and co-workers (1991) also reported that studies incorporating treatments that would influence myofibrillar proteins such as ageing and cooking conditions mainly affected initial yield force values on Warner Bratzler shear-force curves. The peak values reflected the change

related to animal age, muscle type, the amount of connective tissue and also cooking methods.

Factors that affect the results of the WB shear tests and must be controlled are uniformity of sample size (1.27 cm x 1.27 cm), as well as the direction of muscle fibers, the presence of connective tissue and fat deposits, and sample temperature, along with internal temperature. The thickness of the blade and crosshead speed can affect the shear force as well (Zhang and Mittal, 1993).

Another way to objectively measure tenderness is the MIRINZ tenderometer. The MIRINZ tenderometer is similar to the Warner Bratzler shear device, as it assesses tenderness by shearing through meat samples perpendicularly to the fiber direction. It utilizes rectangular samples that are 1.27 cm x 1.27 cm, while the Warner Bratzler was developed using round core samples. (Bouton et al., 1975). Unfortunately, the shear force measurements do not take into account the contribution of moisture and fat content to the sensory perception of juiciness and the impact that is has on the tenderness (Perry, 2001).

Factors Affecting Tenderness

Moeller and Courington, (1998) stated, improving product quality and consistency, with regards to tenderness is an extremely important aspect within the beef industry. Additionally other researchers found taste and tenderness of beef were primary drivers of consumers decisions. This led them to conclude that one of the major problems identified at the consumer level was inconsistency of tenderness (Monson et al, 2004, Peachey et al, 2002, Diezer et al, 1993, Steen et al, 1997, Dikeman, 1987). Variation in

tenderness of meat can be seen within identical muscles of homogeneous animal groups, within like species, within a single breed or breed type, within the same age and sex groups. Tenderness can also be influenced by antemortem factors such as environmental factors like weather and pre-slaughter conditions like stress, along with post-slaughter conditions such as ultimate pH, rate of pH decline or muscle temperature at the time of rigor (Steen et al, 1997).

Vaack, (2000) concluded ultimate pH was a major determinant of meat quality. Knowledge about factors that determine ultimate pH is essential to actively controlling ultimate pH of meat. Glycolytic potential, as a measure of substrate concentration for postmortem glycolysis, is a major determinant of ultimate pH. However, the relationship between glycolytic potential and ultimate pH is far from perfect. The contribution of other factors such as phosphorylase and AMP deaminase enzymes and the possible presence of two glycogen forms need to be evaluated (Vaack, 2000).

Tarrant and Mothersill, (1977) found the time required for the pH to fall to 6.0 varied with the muscle and depth of muscle in the carcass. The rate of ATP turnover and muscle temperature was closely related to temperature ($r=0.97$). These researchers also found smaller pH differences between muscles when evaluating postmortem glycolysis and measuring the rate of ATP turnover compared to muscle temperature. The pH was generally lower in the semitendinosus than in the biceps femoris, and semimembranosus. The researchers concluded these muscle differences were not due to temperature differences, as the temperature differences were small ($<3°C$). These researchers concluded the relationship between pH and temperature may have an important influence

on meat quality. Low pH values (<6.0) corresponded with high temperatures (>30°C). This information supports the idea that the properties of meat that are of extreme interest to the consumer are heavily influenced by treatments and physical conditions that occur in the last few days of life, and in the first few hours after harvest (O'Halloran et al, 1997).

Ultimate Meat pH

Meat tenderness has been shown to be related to the ultimate muscle pH, postmortem temperature, the state of muscle contraction, and the enzymatic proteolysis of myofibrillar proteins (Yu et al, 1986). Interestingly enough, in many production systems where cattle are grown in feedlots, the ultimate pH consistently reached between 5.3-5.7 (Purchas, 1990). Yet variations in tenderness are still reported. The relationship between ultimate pH and tenderness has been shown to have a curvilinear relationship with minimum tenderness found between 5.8 and 6.2 pH values (Purchas 1990; 1993). O'Halloran et al, (1994) stated that high pH meat was more tender than meat of normal pH (5.8-6.2), while meat of high pH (6.3-6.9) was toughest which agrees with Purchase (1990). Yu and Lee, (1986) also reported reduced tenderness with ultimate pH between 5.8 and 6.3 and concluded this reduced tenderness was due to reduced proteolytic activity during the conversion of muscle to meat and postmortem. Finally, Jeremiah et al, (1991) suggested segregation of beef carcasses with ultimate longissimus pH values between 5.8 and 6.19 could be an easy, nondestructive, practical means to effectively remove the majority of tough carcasses in all three sex groups, regardless of breed.

Rate of pH Decline

Meat tenderness has been highly correlated to the rate of pH decline. Many researchers have reported that fast glycolysing muscle had lower shear force values and was rated more tender in sensory panel (O'Halloran et al, 1997, Voisinet et al, 1997, Silva et al, 1999, Yu and Lee, 1986, Hwang et al, 2001, and Shackelford et al, 1994) than steaks from slower glycolysing muscle. O'Halloran et al, (1997) found that faster pH decline or higher glycolytic rate was associated with less tender meat at 2 and 7 days post mortem than meat with intermediate (5.9-6.1) or slow (5.46-5.66) pH decline or glycolytic rate. Additionally, Hwang and Thompson, (2001) showed more tender meat with an intermediate pH decline (5.9-6.2, 1.5 hours postmortem) after 14 days of ageing. After evaluating different rates of pH decline at different times postmortem, Marsh et al, (1987) concluded tenderness was low when glycolysis proceeded at a rate, which produced a pH of 6.1 after 3 hours post mortem when carcass temperatures were between 19.1°C–36.7°C. Marsh et al., (1987) explained this difference in tenderness by postmortem muscles reaching their ultimate pH at warm temperatures greater than 20°C. O'Halloran et al, (1997) reported that the low pH conditions in fast glycolysing muscle enhanced the release of cathepsins B and L from lysosomes. Additionally, calpain I activity was higher and calpastatin was lower in fast glycolysing muscle suggesting a mechanism for increased tenderness in the fast glycolysing muscle. Yu and Lee, (1986) reported the combination of postmortem pH and temperature had significant effect on the structural changes, and degradation pattern of muscle proteins along with final meat tenderness. These researchers reported muscles with postmortem pH ranging from 5.5-

7.0 aged at 1, 4, 25, and 37°C, resulted in muscle with a higher pH exhibiting extensive degradation of the Z-line while muscle with a low pH showed increased degradation of M-line and myosin. Postmortem temperatures of 37°C enhanced degradation of muscle proteins, like myosin heavy chain and the M-Line which suggested the importance of both postmortem pH and temperature for the postmortem degradation of muscle proteins, resulting in greater tenderness. These results strongly suggested that rapid tenderization took place during the early postmortem period (within 24 hr after slaughter) and when the shear was determined after 24 hour chilling period at 4°C, high pH (above 6.3) meat was significantly ($P<0.05$) more tender than low or intermediate pH meat (Yu and Lee, 1986).

Breed

Behrends et al (2009) stated that inheritance is responsible for the variation in cattle performance, especially cattle tenderness. Tatum et al, (1999) found that tenderness comparisons among sires suggested that the rate of nonconformance for strip loin steaks might be reduced by control of genetic inputs into the system. When Campo et al, (1999) evaluated forty-two yearling males from seven European beef breeds they found that ageing time had the most influence on juiciness, and overall flavor intensity. All of the breeds evaluated showed higher tenderness scores as the ageing period increased however, a faster, but shorter and less intense tenderization rate was seen in animals with a double muscled condition., Monson et al, (2004), however, found Warner- Braztler values were significantly different ($P<0.05$) between the breed (Spanish Holstein, Brown Swiss, Limousin, and Blonde d'Aquitaine) after 1, 3, and 7 days of postmortem ageing when evaluating the influence of cattle breed and ageing time on textural meat quality.

The shear force values were lowest in Limousin and Blonde d'Aquitaine, followed by Brown Swiss and Holstein, which were approximately 25% higher. In agreement with Monson et al., (2004), Campo et al, (1999) found significant differences between double muscled, and fast growth breeds (Asturiana de los Walles, Pirenaica, Rebia Gallega, Brown Swiss, Avilena-Negra iberica, Morucha, and Retinta). These researchers showed that double muscled breeds were more tender after one and three days of postmortem ageing ($P<0.01$), but had the lowest tenderness (with a trained eleven member sensory panel) after 21 days ($P>0.05$) of postmortem ageing. This could be explained by the double muscle condition having a different myofibrillar composition with a higher number of muscle fibers, along with different collagen structure with a lower proportion of non-reducible cross links.

Postmortem Ageing

Because tenderness is regarded as a principle determinant of eating quality, a clear understanding of mechanisms of postmortem tenderization is important in order to control tenderness at an industry level (O'Halloran et al, 1994). The increase in muscle tenderness is affected by the function of different proteases. These muscle proteases fall into three categories based upon their optimum pH, which are the alkaline proteases, the neutral proteases and the proteases activated by calcium ions, and the acidic proteases or the cathepsins. Only the proteases that exhibit activity below pH of 7.5 are important during postmortem ageing (Pearson et al, 1983).

Ageing in the meat industry is a popular way of increasing tenderness and decreasing the variation in tenderness among animals. This occurs from the degradation

of myofibrillar proteins and the breakdown of the ultrastructure of the muscle (Beltran et al, 1997; Monson et al, 2004). The improved tenderness during ageing is due to the endogenous muscle proteases that are associated with muscle turnover in the living animal. Monson et al, (2004), stated that increased ageing time eliminated the differences in meat texture between breeds and individuals within the same breed. Additionally, Takahashi, (1996) reported structural weakening of the myofibrils, along with the intermediate filaments, and connective tissues during postmortem ageing and concluded these changes contributed to tenderization of meat. These researchers suggested the increased tenderness due to ageing was from the weakening of Z-disks, weakening of rigor linkages formed between actin and myosin, splitting of titin filaments, and fragmentation of nebulin. Taylor et al, (1995) also found ultrastructural changes during postmortem storage. Electron micrographs of muscle sampled at death, and after 1, 3, and 16 days postmortem (4°C), showed invaginations of the sarcolemma at the level of the Z-disk and the M-line. Koohmaraie, (1992) found that the sarcolemma invaginations (indentations in the surface leading into the interior of the cell) disappeared during the first 24 hours postmortem, and in approximately 50% of the fibers, the sarcolemma was pulled away from the underlying myofibril. These researchers explained this as the breakdown of the costameres (connection point of the sarcomere to the cell membrane) that are responsible for the attachment of the sarcolemma to the myofibril. Furthermore, Taylor et al, (1995) stated that the weakening of the connections between thin filaments in the I-band and the Z-disk was mainly due to degradation of titin and nebulin, which extends into the Z-disk, and are anchored there by interaction with Z-disk proteins.

Olson et al, (1976) demonstrated that the size of muscle fragments that develop during postmortem ageing are muscle and time storage dependent. These researchers isolated myofibrils from bovine muscles at 1, 3, 6, and 10 days postmortem (2°C), and evaluated the light absorption by a spectrophotometer. They found in general, the size of the fiber pieces became progressively smaller with increased postmortem time (increased myofibrillar fragmentation). Myofibrils at day 1 of postmortem were long and contained more than 20 sarcomeres per myofibril, but as postmortem time increased, the myofibrils became more fragmented. Veiseth et al, (2001) reported postmortem tenderization process began approximately three hours postharvest. These researchers suggested the increase in tenderness was due to inter-myofibril linkages like desmin, along with intra-myofibril linkages like titin, and nebulin breaking as time postmortem increased agreeing with data reported by Taylor et al (1995).

Zeece et al, (1986) concluded that a calcium activated factor (CAF) played an extremely important role in the tenderization process. Myofibrils treated with CAF showed a release of soluble protein, which was pH and temperature dependent. Furthermore, during incubation of myofibrils with CAF at optimal conditions, (pH 7.5 and 25°C), a rapid release of soluble protein occurred. However, decreasing the temperature (5°C) of incubation greatly reduced the amount of solubilized protein, but did not completely inhibit the enzyme. Additionally, these researchers found CAF increased the degradation of Z-line and increased myofibril fragmentation in vitro. These researchers reported alpha-actinin appeared to be one of the major components of the solubilized protein released from the myofibrils and that troponin T and tropomyosin,

were degraded more slowly than the other proteins. In agreement with Zeece et al, (1986), Goll et al, (1983) concluded CAF was one of the important agents of postmortem meat tenderization. It was later determined that the calcium activated factor seen in muscle was the calpain system, as calpain was both calcium activated and degraded the proteins from isolated myofibrils similarly to what was seen during postmortem ageing (Goll et al, 1983). Koohmaraie, (1992) hypothesized that the calpain proteolytic system, was responsible for meat tenderization and the degradation of many of the proteins postmortem.

Protein Components Affecting Tenderness

Many researchers have stated that proteolysis of myofibrillar proteins is involved in the improvement of meat tenderness during post mortem storage (Olson et al, 1976; MacBride and Parrish, 1977; Koohmaraie, 1992; Thomson et al, 1996; Campo et al, 1999; Lamare et al, 2002). Negishi et al, (1996), MacBride and Parrish, (1977), Olson et al, (1977), and Parrish et al, (1981) all reported the appearance of a 30 kDa band on SDS-PAGE after postmortem ageing. These researchers observed a reduction in the intensity of the troponin T band with a concomitant increase in the 30 kDa band as time postmortem increased. MacBride and Parrish (1977) found the presence or absence of the 30 kDa component was also related to tenderness. The appearance of the band was seen earlier in tender samples than in less tender samples. These researchers concluded that the appearance of the 30 kDa band could be a predictor of tenderness.

Olson et al, (1977) found when treating isolating myofibrils from longissimus, semitendinosus, and psoas major muscles at death, and at 1,2,3,6 and 10 days postmortem

storage (25°C) with CAF (calpain) that troponin disappeared with an increase in the band corresponding to the 30 kD. Additionally, the disappearance of troponin-T and appearance of 30 kDa component suggested that this 30kDa originated from the degradation of troponin T, along with any myofibrillar proteins having subunit molecular weights larger than 30kDa. After 10 days of postmortem storage, no major changes occurred in the bands of actin, myosin, and alpha-actinin. Goll et al, (1983) stated the principal degradative change detectable in SDS-polyacrylamide gels of postmortem myofibrils was due to a loss of Troponin T. Huff-Lonergan et al, (2010) later confirmed troponin-T was the source of the 30 kDa utilizing antibodies to identify individual bands on the gels. Huff-Lonergan and co-workers (2010) also reported the 30 kDa component was strongly related to the tenderness of beef.

Hwan and Bandman, (1989) reported the rate of desmin degradation in postmortem muscle was similar to the rate of troponin-T degradation and titin degradation. Goll et al, (1983) stated desmin's degradation by CAF (calpains) in postmortem muscle contributed to increased tenderness. Hwan and Bandman, (1989) found desmin was easily degraded at 4°C during the ageing process. Within 96 hours postmortem, fragments of desmin were detected with an antibody. By 3 weeks postmortem, little intact desmin remained in the muscle.

Huff-Lonergan et al, (1995) stated nebulin was degraded postmortem, with a significant difference observed at 3 days postmortem. In less-tender samples the intact nebulin band was still easily detectable 3 days postmortem, however more tender samples displayed only a trace of intact nebulin on SDS-PAGE. This indicated that nebulin

generally was degraded faster in samples that were classified as more tender. Paterson and Parrish, (1987) also found very little nebulin detected in either infraspinatus or the rhomboideus muscles when each was aged 7 days postmortem. Researchers concluded that titin, nebulin, and desmin, but not α-actinin, are degraded postmortem (Ho et al., 1996).

Huff-Lonergan et al, (1996) found when examining purified myofibril (MF) and homogenized whole muscle samples, that intact form of titin (T1) was absent at the same time postmortem in both the purified and whole muscle samples. At early times postmortem (3 days) titin appeared as a distinct doublet, with the lower band, T2 more evident at day 3 than at day 1. However, the T2 band in the MF samples was always faint at day 0. Furthermore, Paterson and Parrish (1987), found differences in titin from the infraspinatus and rhomboideus muscle. These researchers concluded that titin had undergone more complete proteolytic degradation in the tender muscle than in the tough muscle, because of the presence of a more intense protein band for T2 in the tender muscle as compared to a more intense T1 band in the tougher muscles (Paterson and Parrish, 1987) indicating a faster degradation of titin in muscles that were more tender.

Collagen

Marsh, (1977) stated the two muscle components that determine tenderness are collagen and the contractile apparatus. Koohmaraie et al, (2002) added sarcomere length and proteolysis of myofibrillar proteins to connective tissue to account for most of the variation in tenderness. Hall and Hunt, (1982) and Wulf and co-workers (1996) stated collagen is generally believed to influence muscle tenderness as an animal increases in

age. As cattle reach a higher physiological maturity, intramuscular collagen solubility decreases, resulting in increased toughness (Wulf et al, 1996). Increased age resulted in more intermolecular cross-linkages in collagen in meat from cattle fed high energy diets. However, dietary reduction in growth rate was reported to result in lower collagen solubility (Hall and Chadwick, 1982). These researchers reported that cattle fed roughage based diets grew slower and resulted in the animals reaching their mature weight at an older age and the muscle from these animals had lower collagen solubility (Hall and Chadwick, 1982). Additionally, muscles with high collagen content have been known to acquire higher crosslink concentrations per unit of collagen than the muscles with lower collagen content. Increasing age and maturity leads to progressive toughening of meat (Hall and Chadwick, 1982).

Growth Rate

Muscle growth refers to fibers increasing fiber size. During embryonic and fetal development the growth is mainly due to hyperplasia, the increase in number of muscle fibers. The greatest increase in muscle size occurs after birth, yet the rate of this increase declines as the animal reaches maturity (Aberle et al, 2012). Koohmaraie et al, (2002) stated postnatal muscle growth occurs when protein synthesis exceeds protein degradation. This can occurs due to decrease in protein degradation while the protein synthesis level remains the same. Prior to birth, muscle fiber number is set, however, postnatally muscle increases in size from the increase in nuclei in skeletal muscles during growth. This increase in muscle size from increased nuclei is controlled by satellite cells.

Satellite cells, also stimulated in adult muscle in response to injury or exercise (Allen et al., 1983). Hawke and Garry, (2001) stated satellite cells fuse together and combine during postnatal growth, resulting in an increase in muscle fiber size, and therefore muscle growth. Postnatal muscle growth is due to the activation of the satellite cells and their fusion into existing muscle fibers. This increase in DNA available controls protein synthesis (Hawke and Garry, 2001).

Oddy et al, (2001), and Purchas et al, (2002) found rapid growth over the lifetime of an animal often resulted in lower shear force values. Additionally, these researchers stated more rapid growth rate could result in differences in structural and cross linking of the collagen matrix, along with altered proteolytic activity which influences the rate of protein accretion. Purchas et al, (2002) concluded that faster growth rate was associated with more intramuscular fat, ($P<0.05$), and higher myofibrillar fragmentation indexes ($P<0.05$) suggesting faster proteolysis postmortem. However, these researchers found that the differences in tenderness between the three growth-pattern groups (slow, fast and restricted) were small (Purchas et al, 2002).

Temperament

Cattle vary in their behavioral response to stressful events, (Ferguson et al, 2006). Stress is defined as the response of an organism to environmental stimuli that is perceived mostly as a threat (Selye, 1950). Jones, (1983) stated that fear is a crucial component of stress because of the increase in environmental awareness that results in animals under stress which can compound their responses. The stress response normally is comprised of

a cascade of events, which result in an emergency reaction. Following this emergency reaction is the stage of adaptation, and then increased adrenocortical function creates a stage of exhaustion, unfortunately accompanied by disease or death when associated with chronic stress (Selye, 1950). Furthermore, increased adrenocortical activity has been demonstrated in stressed animals (Freeman, and Manning, 1982). Chronic exposure to a variety of stressors can result in hypertrophy and hyperplasia of the adrenal medulla, mainly in the inner zone of the cortical tissue due to increased hormone production (Wells, and Wight, 1971).

Cortisol is known as the principal corticosteroid released in response to stress, and elevated corticosterone levels are seen in response to various stressors. Additionally, a parallel increase in adrenal corticosterone resulted from a stress-induced rate of corticosterone production (Freeman and Manning, 1982). Cortisol, known for coordinating the body's response to stress, activates proteolysis and protein synthesis in the body (Koolman and Roehm, 2005). Additionally, cortisol increases the rate of activity for enzymes involved in gluconeogenesis. Delius et al, (1976) stated that the greater occurrence of fearful behavior in animals resulted in increased adrenocortical activity while Jones, (1983) reported that defense reactions evoked in response to threatening stimuli were inhibited by increased adrenocortical activity. During these fight or flight reactions, mobilization of liver glycogen raised blood glucose level (Freeman, 1983) to help maintain metabolic processes. Blood glucose levels are normally regulated by insulin and glucagon. The glucocorticoids interfere with normal insulin action by promotion of gluconeogenesis (Culbert and Wells, 1975) instead of insulin's normal

function, activation of glycogen synthase. Insulin activates glycogen synthase, inducing several enzymes involved in glycolysis. Excess glucose is stored in the liver in the form of glycogen, where the liver breaks down glucose through glycolysis.

Apple et al (1995) found that subjecting sheep to restraint along with isolation caused a dramatic reduction in the longissimus muscle glycogen reserves prior to slaughter, resulting in an ultimate pH value in excess of 6.0, causing the carcasses to have a dark, dry and firm meat texture. Sanz et al, (1996) reported stressed animals had significantly reduced muscle glycogen level at 48 hours postmortem. While Devine et al (2006), found stress decreased muscle glycogen levels which in turn resulted in elevated ultimate meat pH. These researchers suggested that for optimum meat quality, any extensive on-farm handling of animals should take place as early as possible at a time prior to slaughter so that animals could rest before subsequent lairage and transport to harvest facility.

Researchers found a correlation between enhanced blood lactic acid and temperament. Holmes et al (1973) indicated that more excitable and temperamental animals had higher blood lactate concentrations. Furthermore, these researchers (Holmes et al., 1973) found higher initial values for blood lactic acid associated with more excitable double muscled animals. Due to a reduction in oxidative metabolism in response to stress the utilization of pyruvic acid within the fiber was reduced. This paired with an increase in glycolytic enzyme activity resulted in elevated blood lactate. The same stresses produced a significantly higher percentage of dark cutting (high pH) carcasses (Holmes et al, 1973).

Transportation and handling can affect carcass characteristics, especially meat color. Tarrent, (1990) stated that even under good conditions of transport, cattle manifest physiological and behavioral changes that are indicative of stress. Tarrant et al. (1992) found that when transportation conditions deteriorated, physiological and behavioral changes intensified. Additionally, Villarroel et al, (2003) found that intermediate (3 hour journey) transport time increased the overall liking for steaks by panelists compared to steaks from animals that had short or long journeys (30 min or 6 hours). Data from Tarrant et al (1992) supports Villaroel and co-workers (2003) findings. These researchers stated that as stocking density in the truck increased physiological response to stress along with carcass bruising, and plasma activity of creatine kinase also increased especially as transportation periods got longer. Additionally Tarrant et al. (1992) found that stocking density also affected tenderness and resulted in reduced carcass quality indicating that high density during transport reduced the welfare of the cattle.

When measuring temperament of an animal, Burrow et al, (1988) indicated flight speed, (also known as exit velocity) and chute score or crush score (Australian term for chute score) are two tests, both simple and safe for evaluating temperament. Voisinet and co-workers (1997) used a four point chute score system that was similar to Grandin's (1993) five point system. Grandin, (1993) reported a temperament rating of 1 was a calm animal with no movement in the chute, all the way up to 5 where the animal was rearing, twisting the body and struggling violently in the chute. Grandin (1993) reported chute ratings of 4 and 5 to be behaviorally agitated. Balking ratings were also reported by Grandin (1993). This behavior was described as the rate of balking behavior at the

entrance to the squeeze chute and scale. The animals were either classified as balkers, where a hard slap on the rump or tail twisting was required to induce an animal to enter the chute, or a non-balker, where the animal entered voluntarily. However, as the handling times increased, there was a reduction found in the amount of balking. The chute score data along with balking data was used to compare how the data could be used as a prediction of temperament. Grandin found that agitated bulls, (Gelbvieh x Simmental x Charolais cross bulls) balked less during entry into the squeeze chute or scale. Additionally, cattle also balked less while entering the scale, resulting in an indication of restraint in the squeeze chute was more aversive than the scale (Grandin, 1993). Curley et al, (2006) found measures of exit velocity and chute score to be valuable tools for the assessment of cattle temperament along with a predictor of temperament and stress responsiveness to future animal handling events. Furthermore, Curley et al, (2006) used chute scores to determine that temperament is moderately heritable, indicating there is a genetic contribution to how the animal responds when the animal is handled in the chute.

Behrends et al (2009) reported that the escape velocity tended to be faster at the first measurement, and as cattle become more accustomed to the chute, exited more slowly after more times handled. These researchers suggested that repeated handling, resulted in habituation, which is when animals become more accustomed to the handling procedures and they have reduced movement in the chute and leave the chute at a slower speed (Behrends et al., 2009). This observation was supported by Crookshank et al (1979), who also observed that cattle were less agitated and had reduced cortisol levels after multiple handling experiences. Furthermore, Voisinet and co-workers (1997), along

with Curley et al (2006), reported higher cortisol levels was related to more “agitated” animals, even when there were multiple handling times. This evidence suggests that in cattle there is a direct correlation between levels of cortisol and temperament measurements.

Temperament can vary greatly among breeds. Fordyce et al, (1988) reported that Bos indicus cross cattle had higher temperament scores (temperament scores from 1-7) than Bos taurus cattle. This was in agreement with data presented by Café et al (2010), who found a relationship between temperament and growth as well as an increase in quality grades in carcasses from more docile Angus cattle than carcasses from Brahman cattle. Voisinet et al, (1997) also found Bos indicus animals to have higher temperament ratings along with less tender meat when compared to animals with lower temperament ratings. Furthermore, temperament was associated with differences in tenderness when measured by Warner Bratlzer, ($P<.0001$) after a 14 day ageing period. Researchers found as temperament increased from calm to excitable, WBS increased. These researchers concluded temperament had a significant effect on tenderness (Voisinet et al, 1997).

Fordyce et al, (1988) emphasized the importance of improving temperament through selection for temperament. These researchers used a 7-point scale along with an audible degree of respiration (BLO) score to classify temperament. They concluded that cattle with a more excitable temperament as indicated by a higher temperament score and BLO score had more carcass bruising in the areas of high quality cuts when compared to cattle with less excitable temperament. Additionally, Wolcott et al. (2009) found that temperament traits were moderately heritability and the presence of adequate phenotypic

variation was found in the experimental animals to suggest that temperament could be successfully improved by selection in tropically adapted animals.

Researchers have reported a reduction in growth rate, feed intake and feed efficiency in animals with higher temperament scores. Voisenet et al., (1997) showed a significant effect of temperament ranking on average daily gain in Bos indicus versus Bos taurus cattle, where the Bos taurus steers with calmest temperaments had a greater mean average daily gain than the steers with the higher temperament scores or more excitable temperaments. Café et al, (2010) also reported cattle with a calmer temperament, measured by reduced flight score as well as lower crush scores, had increased growth rate, feed intake and improved feed efficiency. This was supported by data reported by Ferguson et al, (2006) who found cattle with calmer temperaments had improved productivity; however, the effect of temperament on economically important traits was varied.

Some researchers have reported a relationship between temperament and carcass characteristics. Café et al, (2010) found cattle with higher flight scores or chute scores had consistently less feed intake and slower growth rates, which resulted in smaller carcasses with less backfat as well as lower quality grades. This was also supported by Fordyce et al, (1988) who found that the heaviest cattle tended to have the lowest temperament scores, suggesting that selection for higher growth rate may result in milder temperament of animals. This agrees with data reported by Voisinet et al, (1997) and King et al., (2006) who stated that cattle with wilder temperaments, based on chute score and exit velocity, exhibited lower body weight gain. However, King et al, (2006) reported

temperament (average of exit velocity and pen score) did not affect marbling score. Furthermore, Partida et al, (2007) showed that social status relationships did not affect the sensory or instrumental meat quality traits like average conformation scores, fatness scores, tissue composition of the sixth rib, and surface area of the M. longissimus dorsi in young Friesian bulls when evaluating the effect of social dominance on the meat quality.

Consumer purchasing decisions are heavily affected by the palatability of beef products, and several factors including tenderness have been shown to affect this. Consumers have consistently ranked tenderness as the most important attribute to eating quality and overall palatability of beef. Previously stated, tenderness has several factors that affect the final tenderness that most consumers experience. The question of temperament determining, or predicting tenderness values in beef cattle is a very important topic to be explored. Research of temperament, coupled with the chute side convenience of blood lactate meters, can improve the producer to consumer relationship by aiding in the guarantee of tenderness and an improved eating experience for the consumer. Additionally, research investigating whether tenderness is affected by temperament can aid producers in overall selection of sires for their herd. The objective of this study is to determine if temperament effects overall tenderness of beef steak, and can blood lactate be used as an objective measurement of temperament.

3. MATERIALS AND METHODS

Data was collected in compliance with Montana State University Agriculture Animal Care and Use Committee under the Animal Care approval number 2013-AA02. One hundred and fifty four calves born at the Bair Ranch in Martinsdale, Montana as part of the American Simmental Association Carcass Merit Project were evaluated for temperament using chute scores, blood lactate measurements and chute exit velocity measurements. The chute scores, ranging from 1 to 6 were assigned by a single individual at all handling times (Table 1).

Blood lactate concentration was used to assess the muscle response to handling stress. The blood lactate concentration was analyzed with a blood lactate meter (Lactate Pro, Arkray Inc, Minami-Ku, Kyoto, Japan) which uses 5-25 microliters of blood to determine the mmol/L of lactate produced in response to handling stress. Steers were caught in a head gate when they were handled through the chute to be either weighed or vaccinated. A 16 gauge needle was used to pierce the ear vein on the back of the ear to obtain a drop of blood that was placed on the lactate test strip, and analyzed in 60 seconds by the Lactate Pro meter. After blood for lactate measurement and weight were obtained, animals were allowed to leave the chute and the exit velocity was measured with a Farmtek timer system similar to ones used to time roping and barrel racing events. The initial “infrared” gate was placed 1.824 meters in front of the chute and the final gate was 1.824 meters from the initial gate. Exit speed was recorded and the meter per second was calculated for statistical analyses (Curley et al, 2006).

Table 1: Chute Score Descriptions[a]

Chute Score	Definition and Interpretation
Score 1	Docile, mild disposition, gentle and easily handled. Stands and moves slowly during processing. Undisturbed, settled, somewhat dull. Does not pull on head gate when in chute. Exits chute calmly.
Score 2	Restless. Quieter than average, but may be stubborn during processing. May try to back out of chute or pull back on head gate. Some flicking of tail. Exits chute promptly.
Score 3	Nervous. Typical temperament is manageable, but nervous and impatient. A moderate amount of struggling, movement and and tail flicking. Repeated pushing and pulling on head gate. Exits chute briskly.
Score 4	Flighty (Wild). Jumpy and out of control, quivers and struggles violently. May bellow and froth at the mouth. Continuous tail flicking. Defecates and urinates during processing. Frantically runs fence line and may jump when penned individually. Exhibits long flight distance and exits chute wildly.
Score 5	Aggressive. May be similar to Score 4, but with added aggressive behavior, fearfulness, extreme agitation, and continuous movement which may include jumping and bellowing while in chute. Exits chute frantically and may exhibit attack behavior when handled alone
Score 6	Very aggressive. Extremely aggressive temperament. Thrashes about or attacks wildly when confined in small, tight places. Pronounced attack behavior.

[a]Beef Improvement Federation http://www.bifconference.com/bif2012/proceedings-pdf/05Randel.pdf

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 ration 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 with Component TE-S, which contained 120mg of trenbolone acetate and 24 mg of estradiol ESP, along with 29 mg of tylosin tartrate. The temperament was assessed

for all of the steers at weaning (September 9, 2011), at re-vaccination (September 27, 2011), when they were processed into the feedlot (October 31, 2011), and were weighed half way through the finishing phase (January 28, 2012). Average Daily Gain (ADG) was calculated using an adjusted 205 weaning weight and weight at midpoint through the finishing phase. The difference between weight at midpoint through the finishing phase was determined and the gain per day calculated by dividing the difference by the number of days between the measurements (i.e., weight at midway through feedlot phase – adjusted weaning weight / 140 days).

The animals were harvested at the Cargill Beef Processing facility in Fort Morgan, Colorado under federal inspection. Carcass data were collected on all 154 steers using a USDA assisted camera grading system. A subsample of 15 steers with a low average daily gain (ADG) and a subsample of 15 steers with high average daily gain (ADG) from selected sires were chosen and strip loins collected from each carcass from the designated steers. Genetic information using EPD's (expected progeny differences) for tenderness and growth were considered when the steers were selected for subsamples. The loin samples were collected 36 hours after slaughter from the carcasses, and transported to Montana State University (4° C), where loins were cut into steaks and the steaks aged for 3, 7, 14, and 21 days postmortem. After samples were aged, they were frozen for further analyses. The samples collected were used to evaluate shear force tenderness, myofibrillar fragmentation index and protein degradation using SDS-PAGE (sodium dodecyl – polyacrylamide gel electrophoresis).

Shear Force

Steaks for shear force analysis were placed in a cooler at 2°C for 24 hours to thaw, then prior to cooking, steaks were patted dry with a paper towel, and weighed and raw weight recorded. Each steak was placed on a broiler pan, where two copper constantin thermocouples (OMEGA Engineering, INC, P.O. Box 4047, Stamford, Connecticut) were inserted into the geometric center of each steak. The steaks were placed under the broiler (10.16 cm from heating element). The samples were cooked until the internal temperature was 35°C when they were turned and cooked to a final internal temperature of 70°C. When the steaks reached the final internal temperature they were removed from the oven, cooled briefly, and placed in labeled bags and placed in a cooler at 2°C to cool for a minimum of 1 hour. The steaks were then blotted, weighed and the weight recorded. The external fat and outside portion of the steak were removed, and the muscle fiber direction determined. A 1.27 cm slice across the steak surface was made to find the direction of the muscle fibers. A second 1.27 cm thick strip parallel to the fiber direction was cut giving samples that were 1.27 x 1.27 x 2.54 cm. Each core was sheared once perpendicular to the grain, at 200 mm/min with a Food Texturometer (TMS 30 Food Texturometer, Food Technology Corporation, Sterling, VA). Approximately 5-8 cores were sheared per steak and an average of the shear force values was used for statistical analyses.

Myofibril Fragmentation Index

Myofibril fragmentation index was determined following the procedures reported by Culler et al, (1978), as modified by Hopkins et al, (2000). Briefly, 2 grams of sample

were weighed into a cold 50 mL centrifuge tube, where the samples were scissor minced 30 times. Next, 20 mL of cold Standard Salt Solution (SSS, 100mM Potassium Chloride, 20mM potassium phosphate, 1mM ethylene glycol tetraacetic acid (EGTA), 1 mM magnesium chloride, and 1 mM sodium azide) was added to each tube. Each sample was homogenized at 15,000 rpm with a Fisher Scientific Power Gen500 homogenizer for 15 s, and then returned to ice to rest for 15 s. Then each sample was homogenized for an additional 15 seconds at 15,000 rpm, and centrifuged at 1000 x g for 15 minutes (Thermo Scientific Sorvall RC 3P+ Centrifuge, Fisher Scientific). The supernatant was discarded, and the pellet was re-suspended in 5 mL of the SSS then shaken until the pellet was suspended within the solution. Each sample was filtered through a small strainer (approx. 1 mm sieve size in order to remove connective tissue), into cold 50 mL centrifuge tubes, and the strainer washed with an additional 5 mL standard salt solution. The sample was re-suspended and centrifuged three more times, each time decanting supernant and re-suspending in 5 mL cold SSS. After three more washes the pellet was re-suspended in 20 mL of SSS before determining protein concentration following the Biuret procedure. After protein determination the samples were diluted to 0.5 mg/mL and absorbance read at 540 nm. The absorbance was multiplied by 200, resulting in the myofibrillar fragmentation index. Two samples per steer, coupled with duplicate samples, yielded 4 measurements per steer. The average of the MFI (myofibrillar fragmentation index) for each sample (n=2) was calculated, and the average was used for statistical analyses.

Protein Concentration

Protein concentration of the MFI measurements and isolated myofibrils were determined following the Biuret protein determination procedure described by Robson, (1968). A standard curve was constructed in duplicate using known concentration of bovine serum albumin (0, 1.137, 2.274, 4.548, 6.822, 9.096, 11.37 mg/mL). Biuret reagent (Robson et al, 1968) was added, the tubes vortexed and allowed to stand for 15 minutes at room temperature. The optical density was read at 540 nm (Beckman DU 530 Lie Science UV/Vis Spectrophotometer). Unknown protein concentration was determined by placing 0.5 mL of SSS and 0.5 mL of the sample in tubes with Biuret reagent, and0.06 mL of sodium azide. The tubes were let stand for 15 minutes at room temperature. After absorbance was read, the average was calculated and, a linear regression line was constructed in Excel. A typical y=mx+b equation was generated, and the average absorbance added to "b" from the linear regression line from protein concentration, divided by the "m" from the regression multiplied by two (dilution into the tubes). This determined the concentration, which aided us to find the mL of sample needed to meet the concentration requirements and the mL of SSS was determined by subtracting mL sample from 5 for MFI determination.

Myofibril Isolation

Myofibrils were isolated following the procedures of Goll et al., (1974) as modified by Boles et al., (1991). Samples (3±0.05 g) were weighed and placed in labeled cold 50 mL centrifuge tubes, and 30mL of SSS were added to each tube. Samples were homogenized for 10 seconds using a Fisher Scientific Power Gen500 (set at 4,

approximately 15,000 rpm). Samples were centrifuged at 1000x g for 10 minutes in (Thermo Scientific Sorvall RC 3P+ Centrifuge). After centrifugation, the supernatant was discarded, and the pellet was suspended in 18 mL SSS, and homogenized for another 5 seconds. Samples were returned to centrifuge for 10 minutes at 1000 x g after which the supernatant was discarded. The pellet was suspended in 24 mL SSS and homogenized for 5 seconds, and the sample filtered to remove connective tissue (1.0 mm mesh) into centrifuge tubes. Samples were centrifuged for 10 minutes at 1000 x g, and the supernatant was discarded. The previous step was repeated. Sample pellet was suspended in 18 mL 1% Triton (Triton x-100 Fisher Scientific) in SSS. Samples were homogenized for 5 second and centrifuged at 1500 x g (Thermo Scientific Sorvall RC 3P+ Centrifuge), after which the supernatant was discarded. The previous step was repeated. The pellet was re-suspended in 24mL SSS and homogenized for 5 seconds with the Fisher Scientific Power Gen500set on 3. The sample was centrifuged for 10 minutes at 1500 x g, and the supernatant discarded. The pellet was suspended in 24 mL of 100 mM Potassium Chloride, and centrifuged for 10 minutes at 1500 x g, and supernatant was discarded. The previous step was repeated. The pellet was suspended in 30 mL 1.5 M Tris-HCl, (pH 8.0), The sample was then transferred to stronger centrifuge tubes, and centrifuged for 10 minutes at 3000 x g, (Jouan AFAQ ISO 9001 GR 2022) and the supernatant was discarded. The pellet was suspended and the previous step repeated. The final pellet was suspended in 12 mL of 5 mM Tris-HCl for protein determination.

For gel sample preparation, after determining protein concentration, the amount of sample was calculated using the following equation: X mL of sample to add to vial –

(Desired concentration) x (Total volume)/ (Actual Conc. of Sample), and 1- X mL of sample- y mL of Tris. Next, samples were diluted (0.5 mg/mL) and combined with 5 mM Tris-HCl in microfuge tube properly labeled with sample and date. Tracking dye was added [5% (wt/vol) SSS, 50% (wt/vol) sucrose, 0.05% (wt/vol) bromophenol blue, and 5 mM 2-N-morphinoethanesulfonic acid (MES) (pH 6.5)] and 0.1 mL of MCE (2-mercaptoenthenol) The sample was heated at 60°C for 20 minutes, and store at -80°C until loaded on gels.

SDS- Polyacrylamide Gel Electrophoresis

SDS-Page was conducted according to the procedure of Laemmli (1970) with modifications to accommodate the different molecular weight proteins as reported by Boles et al., (1999). The amount of protein loaded onto each gel was 20 µg. Gels containing 4% (w/v) (stacking gel) and 10% (w/v) (separating gel) acylamide were prepared from a stock solution of 30% (w/v) acrylamide (37:1, acrylamide: N,N'-bis-methylene acrylamide). The final concentrations in the separation gels were as follows: 0.375 M Tris-HCl (pH 8.0), 0.1% SDS, and 2mM EDTA. The gels were polymerized chemically by the addition of 0.025% tetramethylethlenediamine (TEMED) and ammonium persulfate. The 4% acrylamide stacking gel contained 0.375 M Tris-HCl (pH 6.7), 0.1% SDS and 2mM EDTA. Changes in titin and nebulin were monitored using a 5% acrylamide (100 acrylamide:1 N,N'-bis-methylene acrylamide) gel containing 0.375 M Tris-HCl (pH 8.0), 0.1% SDS, and 2 mM EDTA. The dimensions of the gels were 160 mm x 180 mm. Gels were run at 35 mA. Gels were stained overnight in a 0.1% (w/v) Coomasie brilliant blue R-250, 10% glacial acetic acid, and 50% methanol, and then were

destained in an excess of the same solution excluding the Coomasie brilliant blue. Gels were imaged with a Bio-Rad ChemiDoc XRS+ machine with Image Lab software (Bio-Rad Laboratories, Hercules, CA). Band recognition analysis was used to visualize dense protein bands that appear in each lane to identify each of the proteins located on the gel.

Statistical Analysis

The experimental units were the individual steers. Growth and carcass data were collected on each individual steer harvested and the analysis of that data was calculated on 154 steers. Tenderness data were obtained from thirty subsampled steers. The GLM model of SAS was used to analyze differences in growth and carcass data, where the class variables were the animals, growth classification, speed classification and lactate classification. The dependent variables were average daily gain and carcass characteristics. To identify the growth, speed and lactate classifications the mean and standard deviation was calculated using 156 animals for all temperament measurements. The means plus one standard deviation were designated “high” growth, “fast” speed or “high” lactate, and the mean minus one standard deviation represented “low growth, slow speed or low lactate. The rest were designated as medium for the classification. The GLM procedure of SAS was also used to analyze tenderness data (MFI and shear force). The classifications described above were used along with days aged as the independent or class variables, and dependent variables were shear force and MFI values. Interactions between days of ageing and growth, speed and lactate class were also tested. The LSMEANS procedure of SAS was used to separate means. Pearson correlations were

calculated on all data. Data were considered significant when the P-value was less than or equal to 0.05.

4. RESULTS AND DISCUSSION

Relationship Between Temperament Measurements

The average chute scores and exit velocities indicated the animals tended to be restless to nervous when handled. Very few were wild with none of the animals evaluated being aggressive (Table 2). Lactate measurements generally ranged from below detectable limits (0.8 mmol/L) to 11.3 mmol/L. The average lactate measurements suggested the majority of the animals when handled had blood lactate concentrations between 2.5 mmol/L and 3.0 mmol/L (Table 2). Exit velocities reported here are slightly lower than those reported by Behrends et al. (2009) and chute scores and exit velocity reported here are similar to numbers reported by Café (2011) for Brahman cattle. Exit velocities generally were reduced as the number of handling times increased, indicating a possible "learning" effect or increased comfort with the process (Table 3). Café (2011) also, observed a slight reduction in exit velocity and chute score as the number of handling experiences increased. Even though our docility scores were based on the 1-6 BIF chute score system (Table 1), our maximum score was only 4 with none of the animals exhibiting aggressive behavior. Therefore, this raises the question of whether or not the docility scores are a good measurement of temperament or, if exit velocity and lactate measurements are better measures and do these have a significant effect on tenderness.

Simple correlation coefficients between chute score, exit velocity, blood lactate concentration and ADG at the four handling times are displayed in Table 3. Correlations

between temperament measurements and lactate were low but significant at all measurement times. Correlations between average daily gain and measurements of temperament or blood lactate were not significant at any of the times measured. This disagrees with data reported by other researchers (Café et al., 2011; Behrends et al., 2009; Voisinet et al., 1997) who found a reduction in average daily gain with more excitable animals. The level of significance for the relationships was reduced at the later measurement times. Café et al. (2011) also observed a reduction in the correlation coefficients with multiple measurements. The low correlation coefficients between exit velocity and chute score reported here (Table 3) for measurements made at weaning and re-vaccination are similar to those reported by Café et al. (2011). Correlation coefficients between lactate and chute score are low ($r=0.32$) but significant ($P=0.0002$) at weaning but the relationship between chute score and blood lactate concentration is reduced the more times the animals were handled. This could suggest two different avenues of interpretation. One would be that the increased exposures to handling made the animal more comfortable with the environment resulting in less stress response. The second option is the steers learn to fight the head chute less resulting in lower subjective scores that are not sensitive enough to reveal the anaerobic stress and concomitant increase in lactic acid production the animal is actually experiencing. The latter option would be supported by the higher correlation between exit velocity and lactate levels at the later measurement times.

Table 2: Simple means of temperament measurements and growth data.

Variable	N	Mean	Maximum	Minimum	Std Dev
Chute Score					
Weaning	154	2.44	4.00	1.00	0.88
Re-vaccination	153	2.58	5.00	1.00	0.81
Feedlot Entrance	153	2.25	4.00	1.00	0.85
Midway Feedlot	154	2.65	4.00	1.00	0.64
Ave. Chute Score[a]	154	2.48	3.75	1.25	0.51
ADG [b]	151	3.40	4.48	1.97	0.47
Exit Velocity					
Weaning	120	3.24	5.36	0.94	0.86
Re-vaccination	142	3.33	6.42	0.97	0.98
Feedlot Entrance	152	2.80	7.17	0.66	1.06
Midway Feedlot	153	2.30	4.93	0.24	0.87
Exit Velocity AVG[a]	154	2.88	4.99	1.04	0.77
Blood Lactate					
Weaning	125	2.61	7.8	0.80	1.51
Re-vaccination	152	3.06	9.1	0.80	1.63
Feedlot Entrance	154	2.92	11.30	0.80	1.73
Midway Feedlot	154	2.59	10.20	0.80	1.76
Lactate AVG[a]	154	2.81	7.72	1.00	1.09

[a] Average blood lactate, average exit velocity, and exit chute score are the average of the four measurements taken at weaning, re-vaccination, feedlot entrance, and midway feedlot.

[b] Average Daily Gain = (feedlot weight-weight – adjusted 205 weaning weight)/140 days

Effect of Temperament and Growth Classification on Carcass Characteristics

Temperament and growth classification significantly affected selected carcass characteristics. The ADG in each growth classification was significantly different indicating our method of classification was successful at separating the groups. As expected, faster growing animals resulted in significantly larger carcasses with a larger ribeye area than did either the medium or slow growing animals (Table 4). This is supported by data reported by Karlsson et al, (1993), who reported growth performance was highly correlated to carcass weights with faster growing animals resulting in larger carcass weights. This could easily be explained by the higher growth rate creating a larger animal, resulting in a larger carcass weight. This observation was also supported by Koohmaraie et al, (2002) who stated muscle growth occurred when protein synthesis exceeds protein degradation indicating that increased muscle accretion is expected with faster growth rates and larger carcass weights. Exit classification showed a strong trend to impact carcass weights. The animals that left the chute more slowly possessed lower carcass weights than the steers that left the chute at a fast or medium rate. Café and co-workers (2011), however, concluded that cattle with more excitable temperaments, as measured by flight score (exit velocity) and chute score, had less feed intake and slower growth rate than cattle with less excitable temperaments which would result in reduced carcass weights. Voisinet et al., (1997) along with Behrends et al., (2009) also found a decrease in growth rate as measured by average daily gain in animals with more excitable

temperament. One explanation for the difference in our data is the steers used for this experiment were Bos taurus animals, whereas the other researchers

Table 3: Simple correlation coefficients (P-value) between chute scores, exit velocity and lactate values measured at four times and average daily gain (ADG2) over the period between weaning and mid feedlot measurement.

	Chute Score	**Exit Velocity**	**Lactate**	**ADG**
Weaning				
Chute Score	1.0			
Exit Velocity	0.47 (<0.0001)	1.0		
Lactate	0.32 (0.0002)	0.31 (0.0007)	1.0	
ADG[a]	-0.06 (0.43)	-0.1 (0.88)	-0.11 (0.21)	1.0
Re-vaccination				
Chute Score	1.0			
Exit Velocity	0.31 (0.0002)	1.0		
Lactate	0.14 0.08)	0.27 (0.001)	1.0	
ADG[a]	-0.002 (0.97)	-0.03 (0.73)	-0.04 (0.63)	1.0
Feedlot Entrance				
Chute Score	1.0			
Exit Velocity	0.65 (<0.0001)	1.0		
Lactate	0.17 (0.04)	0.26 (0.0014)	1.0	
ADG[a]	0.04 (0.63)	0.08 (0.35)	-0.04 (0.62)	1.0
Midway Feedlot				
Chute Score	1.0			
Exit Velocity	0.05 (0.50)	1.0		
Lactate	0.15 (0.05)	0.26 (0.0014)	1.0	
ADG[a]	-0.04 (0.63)	0.04 (0.60)	-0.15 (0.06)	1.0

[a]ADG = (feedlot weight – weaning weight)/140 days

looked at either Bos indicus animals alone or in comparison to Bos taurus animals. This suggests that responses to stress as measured by exit velocity and chute scores may may be different in Bos indicus when compared to Bos taurus.

Relationship of Temperament Measures to Carcass Characteristics

When evaluating the simple correlation between temperament measures and carcass data for all steers (n=154) several significant differences were observed. As would be expected with the significant effect of growth rate on carcass weight, ADG was significantly correlated with carcass weight (P-value <0.0001). Furthermore, carcass weight was significantly correlated to ribeye area (P<0.0001) but not fat thickness or yield grade. Boles et al., (2009) reported increased hot carcass weight with an increased ribeye area with cattle sired by Continental bulls when compared to cattle sired by Angus bulls. This confirms the relationship between ribeye area and carcass weight discussed above. As expected, the carcass characteristics that are used to calculate yield grade were significantly correlated to yield grade. Fat thickness (P<0.001) was positively correlated to yield grade. This indicated that as external fat thickness increased the yield grade also increased. Ribeye area (P<0.001), however, was negatively correlated to yield grade, showing a decrease in yield grade as the ribeye area increased. As expected, fat thickness was positively correlated to marbling scores indicating that as external fat increased there was also an increase in marbling in the ribeye. Furthermore, our results showed that growth rate was not related to marbling (P=0.909). This data is in contrast to Purchas et

al, (2002) who concluded that faster growth rate was associated with more intramuscular fat ($P<0.05$).

Table 4: Effect of growth rate, exit speed, and blood lactate on carcass characteristics (least squares means)

Class	ADG[1]	Carcass Weight(kg)	Marbling Score[2]	Ribeye area (cm^2)	Fat Thickness (cm)	Yield Grade
Growth Rate[3]						
Fast	4.05[a]	398.7[a]	488.4	88.3	0.9[b]	2.8[a]
Medium	3.47[b]	384.0[b]	511.5	84.0	1.3[a]	3.2[a]
Slow	2.63[c]	371.2[c]	485.8	83.2	1.1[ab]	2.9[b]
P-Value	<0.0001	<0.0001	0.41	0.08	<0.0001	0.002
Exit Speed[3]						
Fast	3.5[a]	386.8	502.9	85.0	1.1	3.0
Medium	3.4[a]	390.2	494.1	85.7	1.1	3.0
Slow	2.5[b]	376.9	488.7	84.9	1.1	2.9
P-Value	0.01	0.06	0.75	0.90	0.63	0.82
Blood Lactate[3]						
High	3.4	386.2	507.8	85.1	1.0	3.0
Medium	3.4	386.1	495.6	86.9	1.1	2.9
Low	3.3	381.5	482.3	83.4	1.2	3.1
P-Value	0.39	0.69	0.67	0.16	0.50	0.47

[1] ADG=(feedlot weight – 205 adjusted weaning weight)/140
[2]Marbling scores: 300 = slight, 400 = small, 500 = modest, 600 = moderate
[3]Classifications for growth rate, exit speed and blood lactate are based on ± one standard deviation from the mean. Plus one standard deviation is fast or high and minus one standard deviation is low. The rest of the steers were classified as medium.
[a,b,c] means within a column with different superscripts are significantly different $P<0.05$

Temperament measurements were not correlated with most carcass characteristics with the exception of a strong trend for fat thickness to be negatively correlated with average blood lactate (P-value= 0.06) and exit speed (P=0.08). This indicated that as the average blood lactate and exit speed increased, fat thickness decreased. There could be several explanations for these trends. One explanation could be that animals that are more

"temperamental" are more active and burn more energy thus, less fat is deposited as stored energy. Another potential explanation is that temperamental cattle may have higher or more frequent peaks of cortisol that alter metabolism resulting in less energy stored as fat.

Effect of Growth, Exit Speed and Blood Lactate Classification on MFI and Shear Force Values

When examining the effect of growth classification on MFI and shear force, faster growth rate significantly effected shear force values but had no effect on MFI values. Steaks from faster growing animals were significantly more tender than steaks from slow growing animals (Table 5). These results are supported by Oddy et al., (2001), and Purchas et al., (2002) who reported that rapid growth over the lifetime of an animal often resulted in lower shear force values. This could be explained by more rapid growth rate resulting in differences in structural and cross linking of the collagen matrix, along with altered proteolytic activity which influences the rate of protein accretion. Furthermore, Goll et al. (1992; 1998) indicated an increased calpain activity increased rate of muscle protein degradation, decreased protein accretion and increased tenderness postmortem. Blood lactate classification also significantly affected shear values (P=0.02). The steaks from steers with medium blood lactate levels were significantly more tender than steaks from steers with high or low blood lactate. This could indicate that low and high levels of lactate in the muscle ante mortem altered the rate of pH decline enough to impact

Table 5: Simple correlations coefficients between (P-value) between average chute score, average exit velocity and average blood lactate values and carcass measurements.

	Carcass weight	**Marbling**	**Fat thickness**	**Ribeye area**	**Yield grade**	**ADG[1]**	**Average docility score[2]**	**Average exit score[2]**	**Average blood lactate[2]**
Carcass weight	1	0.15 (0.07)	-0.10 (0.21)	0.42 (<0.0001)	0.01 (0.9)	0.34 (<0.0001)	0.14 (0.08)	0.13 (0.11)	0.07 (0.42)
Marbling		1	0.21 (0.01)	0.08 (0.32)	0.10 (0.20)	-0.01 (0.91)	0.04 (0.62)	-0.02 (0.82)	0.02 (0.80)
Fat thickness			1	-0.32 (<0.0001)	0.74 (<0.0001)	0.05 (0.55)	-0.13 (0.12)	-0.14 (0.08)	-0.15 (0.06)
Ribeye area				1	-0.78 (<.0001)	0.08 (0.37)	0.03 (0.78)	0.05 (0.59)	0.05 (0.57)
Yield Grade					1	0.09 (0.24)	-0.03 (0.71)	-0.06 (0.47)	-0.09 (0.26)
ADG[a]						1	0.08 (0.35)	0.05 (0.56)	-0.10 (0.21)
Average docility							1	0.66 (<0.0001)	0.48 (<0.0001)
Average exit score[b]								1	0.38 (<0.0001)
Average blood lactate[b]									1

[1]ADG= (feedlot weight-weaning weight)/140 days

[2]Average blood lactate, average exit velocity, and exit chute score are the average of the four measurements taken at weaning, re-vaccination, feedlot entrance, and midway feedlot.

tenderness. This is supported by multiple researchers (O'Halloran et al, 1997, Voisinet et al, 1997, Silva et al, 1999, Yu et al, 1986, Hwang et al, 2001, and Shackelford et al, 1994) who reported meat tenderness is influenced by rate of pH decline. Numerous researchers have reported that fast glycolysing muscle had lower shear force values and was rated more tender in sensory panel than steaks from slower glycolysing muscle (O'Halloran et al, 1997, Voisinet et al, 1997, Silva et al, 1999, Yu and Lee, 1986, Hwang and Thompson, 2001, and Shackelford et al, 1994). Additionally, Marsh et al., (1987) explained the difference in tenderness caused by rate of pH decline as the muscle reaching ultimate pH at warmer carcass temperatures. A strong trend was also noted for

Table 6: Effect of growth, exit speed and blood lactate classification on myofibrillar fragmentation index (MFI) and shear force values (least squares means).

Class	MFI	Shear
Growth Rate[1]		
Fast	41.4	55.7[b]
Slow	37.4	66.0[a]
P-Value	0.16	0.02
Exit Speed[1]		
Fast	39.6	69.3
Medium	42.1	60.5
Low	36.5	55.7
P-Value	0.27	0.07
Blood Lactate[1]		
High	39.5	57.1[b]
Medium	39.7	67.9[a]
Low	39.5	60.5[ab]
P-Value	0.97	0.02

[1]Classifications for growth rate, exit speed and blood lactate are based on ± one standard deviation from the mean. Plus one standard deviation is fast or high and minus one standard deviation is low. The rest of the steers were classified as medium.
[a,b] means within a column with different superscripts are significantly different $P<0.05$

exit speed on shear values. On average steaks from animals that left the chute at a fast speed tended to have higher shear force values than steaks from steers leaving the chute at a medium or slow velocity. This agrees with the work of Café et al, (2011), Voisinet et al, (1997), and Behrends et al, (2009) which indicated that steaks from cattle with more excitable temperaments as measured by chute score and exit velocity, had higher shear force values along with a concomitant increase in dark cutters.

The data is presented in figures 1 – 3 for evaluation purposes only. There were no significant interactions between postmortem ageing time and growth rate, exit speed or lactate classifications. The graphs indicate that there are numeric differences in shear force values between classifications for growth rate, exit velocity and blood lactate early postmortem. These differences decrease as ageing time increases. As can be seen in the graphs there is a greater difference in the shear values after three days of postmortem ageing than after 14 or 21 days. This indicates the meat is becoming more tender with increased postmortem ageing and that most of the tenderness differences between the different classifications are gone by 14 days postmortem.

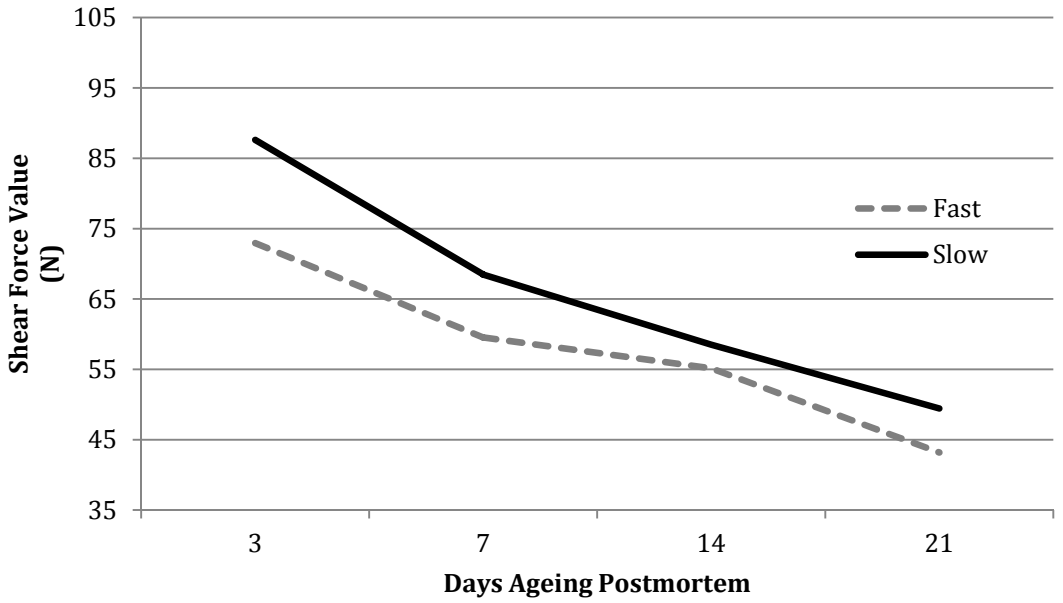

Figure 1: Interaction of growth classification and postmortem ageing on shear force values. Classification for growth rate was based on ± one standard deviation from the mean. Plus one standard deviation is fast minus one standard deviation is low.

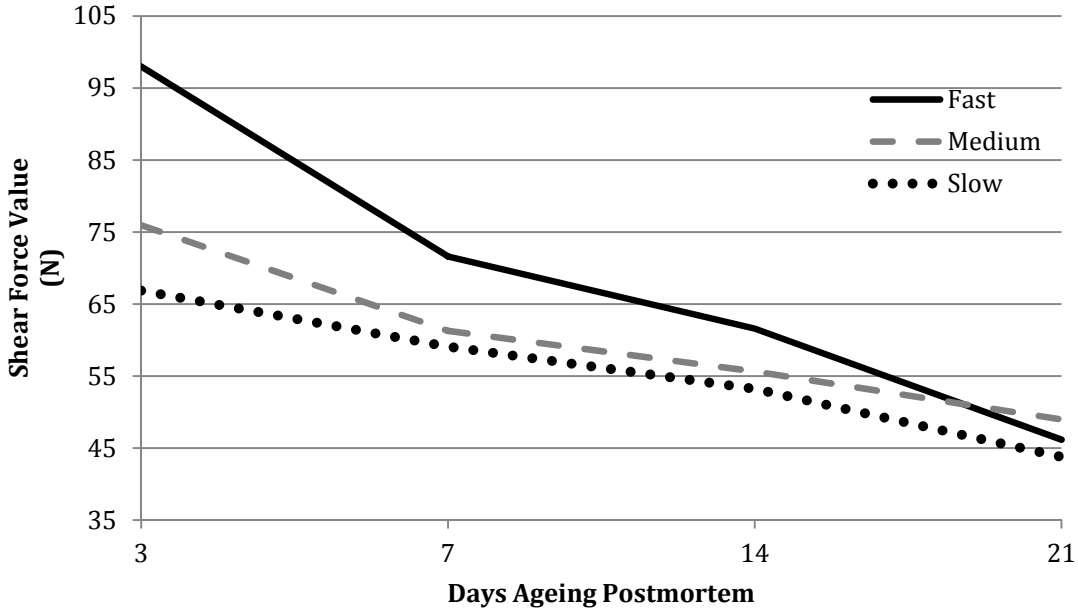

Figure 2: Interaction of exit speed classification and postmortem ageing on shear force values. Classification for exit speed was based on ± one standard deviation from the mean. Plus one standard deviation was fast, minus one standard deviation was slow and the rest were medium.

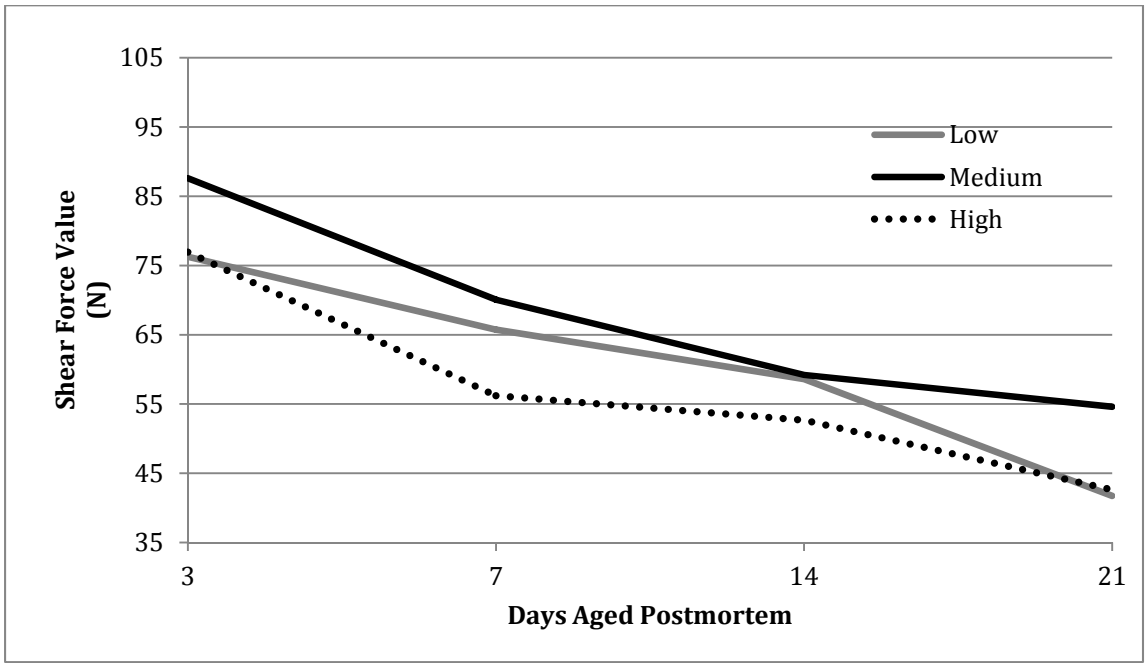

Figure 3: Interaction of blood lactate classification and postmortem ageing on shear force values. Classification for lactate concentration was based on ± one standard deviation from the mean. Plus one standard deviation was high, minus one standard deviation was low and the rest were medium.

Relationship Between Carcass and Tenderness Measurements

There was a significant negative correlation between carcass weight and shear force after 3 days (P=0.02) and 7 days (P=0.05) of postmortem ageing and a negative correlation between ADG and shear force after 3 days of postmortem ageing (Table 7). This may suggest faster growing animals with larger carcasses will be more tender (lower shear force values) especially with less postmortem ageing. This was supported by Oddy et al, (2001), and Purchas et al, (2002), who found more rapid growth over the lifetime of an animal often resulted in lower shear force values. Furthermore, Voisinet et al, (1997) and King et al., (2006) reported that cattle with wilder temperaments, based on chute score and exit velocity, exhibited lower body weight gain and produced less tender meat.

However, there was a positive correlation between marbling score and shear force value after 21 days of ageing (Table 7) suggesting that higher quality grades were resulting in higher shear force values (P=0.02).

Relationship Between Temperament Measurements and Tenderness

Temperament measurements were not strongly related to tenderness. However, a significant correlation (P=0.04) was observed between the average exit speed and the shear force values after 3 days of postmortem ageing. This suggests that steers with a faster exit speed resulted in meat with higher shear force values. Additionally, exit speed was correlated to docility measurement (P=0.001) and lactate concentrations. This was supported by (Voisinet et al, 1997) who found as temperament increased from calm to excitable, Warner-Bratzler shear force values increased, therefore, concluding temperament had a significant effect on tenderness (Voisinet et al, 1997).

As expected a significant correlation (P – value $\leq$ 0.02) was seen between MFI values after 7, 14 and 21 days of postmortem ageing (Tables 7 & 8). This indicated that if there is a high MFI value after 7 days of postmortem ageing there was a higher value after 14 and 21 days of ageing. Furthermore, there was a significant relationship (P – value $\leq$ 0.03) between the shear force values (Tables 7 & 8). If the shear force values were high after 3 days of postmortem ageing they were higher after 7, 14 and 21 days of ageing. This suggests that even if shear force values become more similar with postmortem ageing, and if they are higher after 3 days of ageing, it will be higher after 21 days of ageing, again contributing to variation in samples in the marketplace.

Table 7: Simple correlations coefficients between (P-value) carcass and tenderness measurements.

	CWT[1]	MARB[1]	FAT[1]	REA[1]	YG[1]	ADG[1]	MFI3[1]	MFI7[1]	MFI14[1]	MFI21[1]	SHR3[1]	SHR7[1]	SHR14[1]	SHR21[1]
CWT[1]	1	0.05 (0.81)	-0.34 (0.04)	0.29 (0.11)	-0.02 (0.93)	0.60 (0.0005)	0.06 (0.74)	0.20 (0.30)	0.06 (0.76)	0.25 (0.19)	-0.41 (0.02)	-0.36 (0.05)	0.02 (0.92)	-0.16 (0.39)
MARB[1]		1	0.28 (0.13)	0.05 (0.78)	0.09 (0.62)	0.18 (0.34)	-0.12 (0.51)	-0.20 (0.29)	-0.06 (0.75)	-0.01 (0.98)	-0.12 (0.52)	0.18 (0.33)	-0.007 (0.96)	0.40 (0.02)
FAT[1]			1	-0.42 (0.02)	0.61 (0.0003)	-0.003 (0.99)	0.11 (0.55)	-0.11 (0.55)	-0.08 (0.96)	-0.01 (0.96)	0.03 (0.86)	0.10 (0.59)	0.05 (0.81)	0.09 (0.63)
REA[1]				1	0.90 (<0.0001)	0.22 (0.25)	0.04 (0.85)	0.004 (0.98)	-0.008 (0.96)	-0.16 (0.39)	0.02 (0.93)	-0.01 (0.94)	0.11 (0.55)	-0.14 (0.45)
YG[1]					1	0.04 (0.83)	-0.33 (0.86)	0.02 (0.91)	-0.02 (0.92)	-0.01 (0.95)	-0.15 (0.44)	-0.08 0.68	-0.05 (0.77)	0.11 (0.58)
ADG[1]						1	0.07 (0.70)	-0.02 (0.89)	-0.10 (0.60)	0.01 (0.94)	-0.38 (0.04)	-0.20 (0.28)	-0.10 (0.60)	-0.11 (0.56)
MFI3[1]							1	0.30 (0.10)	-0.24 (0.20)	0.07 (0.70)	-0.32 (0.09)	0.005 (0.98)	-0.32 (0.09)	0.33 (0.08)
MFI7[1]								1	0.28 (0.13)	0.42 (0.02)	-0.29 (0.11)	-0.41 (0.02)	-0.56 (0.001)	-0.38 (0.04)
MFI14[1]									1	0.62 (0.0003)	0.05 (0.81)	0.02 (0.92)	-0.19 (0.34)	-0.03 (0.90)
MFI21[1]										1	-0.14 (0.46)	-0.11 (0.53)	-0.33 (0.08)	-0.22 (0.23)
SHR3[1]											1	0.69 (<0.0001)	0.31 (0.10)	0.39 (0.03)
SHR7[1]												1	0.33 (0.07)	0.53 (0.002)
SHR14[1]													1	0.26 (0.17)
SHR21[1]														1

[1]CWT = hot carcass weight, MARB = USDA marbling score, FAT = fat thickness, REA= ribeye area, YG= yield grade, ADG= average daily gain, MFI= myofibrillar fragmentation index, aged postmortem 3, 7, 14 and 21 days, SHR= shear force value, aged postmortem 3, 7, 14 and 21 days

Table 8: Simple correlations coefficients between (P-value) average docility scores, exit velocity and blood lactate of steers and carcass tenderness measurements.

	DAVE	SPEED	LAVE	MFI3	MFI7	MFI14	MFI21	SHR3	SHR7	SHR14	SHR21
DAVE	1	0.57 (0.001)	0.59 (0.0006)	0.26 (0.16)	-0.04 (0.82)	-0.25 (0.18)	0.25 (0.20)	0.15 (0.44)	-0.11 (0.58)	-0.10 (0.61)	-0.24 (0.20)
SPEED		1	0.46 (0.01)	0.12 (0.53)	-0.10 (0.61)	0.36 0.17	-0.11 (0.57)	0.38 (0.04)	0.10 (0.60)	0.14 (0.45)	-0.04 (0.84)
LAVE			1	0.23 (0.22)	-0.09 (0.62)	-0.09 (0.65)	-0.06 (0.73)	0.16 (0.39)	0.005 (0.97)	-0.005 (0.98)	-0.12 (0.53)
MFI3				1	0.30 (0.10)	-0.24 (0.20)	-0.07 (0.70)	-0.32 (0.09)	-0.41 (0.02)	-0.32 (0.09)	-0.33 (0.08)
MFI7					1	0.28 (0.13)	0.41 (0.02)	-0.30 (0.11)	0.43 (0.02)	-0.56 (0.001)	0.38 (0.04)
MFI14						1	0.62 (0.0003)	0.05 (0.81)	0.02 (0.92)	-0.19 (0.32)	-0.02 (0.89)
MFI21							1	-0.14 (0.46)	-0.12 (0.54)	-0.32 (0.08)	-0.22 (0.23)
SHR3								1	0.70 (<0.0001)	0.31 (0.09)	0.39 (0.03)
SHR7									1	0.33 (0.07)	0.53 (0.002)
SHR14										1	0.26 (0.17)
SHR21											1

[1]DAVE= average docility score, SPEED= average exit velocity measurement, LAVE= average blood lactate, MFI= myofibrillar fragmentation index, SHR= shear force value

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Visualization of Protein Degradation

Using the Image Pro software band recognition tool, 10% and 5% gels were analyzed to compare isolated myofibrils from muscle samples from steers. Results from the 5% gels were used to determine the degradation of larger molecular weight proteins. The categories that were compared on 10% and 5% single gels were a) fast growth rate compared to slow growth rate, b) tender compared to tough samples, and c) fast growth rate with high blood lactate concentration compared to fast growth rate with low blood lactate concentration. Samples from eight steers in each category were evaluated. The myofibrils were isolated from muscle from steers that were categorized as the "extreme" samples from the 30 subsampled steers based on data collected on live animals and steaks from the live animals. Tough and tender category was based on 3 day shear force measurements.

Figures 4 and 5 shows representative 10% and 5% gels of isolated myofibrils with fast growth rate on the left and slow growth rate on the right. The myofibrils were isolated from steaks aged 3, 7, 14, and 21 days (4°C) from steers that had high and low ADG in the early feedlot period. No differences in the banding patterns were observed between myofibrils isolated from fast and slow growing animals.

Figures 6 and 7 show representative 10% and 5% gels of isolated myofibrils with lower shear force values after 3 days of postmortem ageing or more tender samples on the left and less tender samples on the right. The myofibrils were isolated from steaks aged 3, 7, 14, and 21 days (4°) with low or high average shear force values at 3 days

postmortem. No differences in the banding patterns were observed between the tender and tough samples.

Figure 8 and 9 show representative 10% and 5% gels of isolated myofibrils with high blood lactate concentration and fast growth rate based on ADG in the feedlot on the left, compared to low blood lactate concentration and high growth rate on the right. The myofibrils were isolated from steaks aged 3, 7, 14, and 21 days at 4°C. No differences in the banding patterns were observed between the fast growth rate and high blood lactate versus the fast growth rate and low lactate animals.

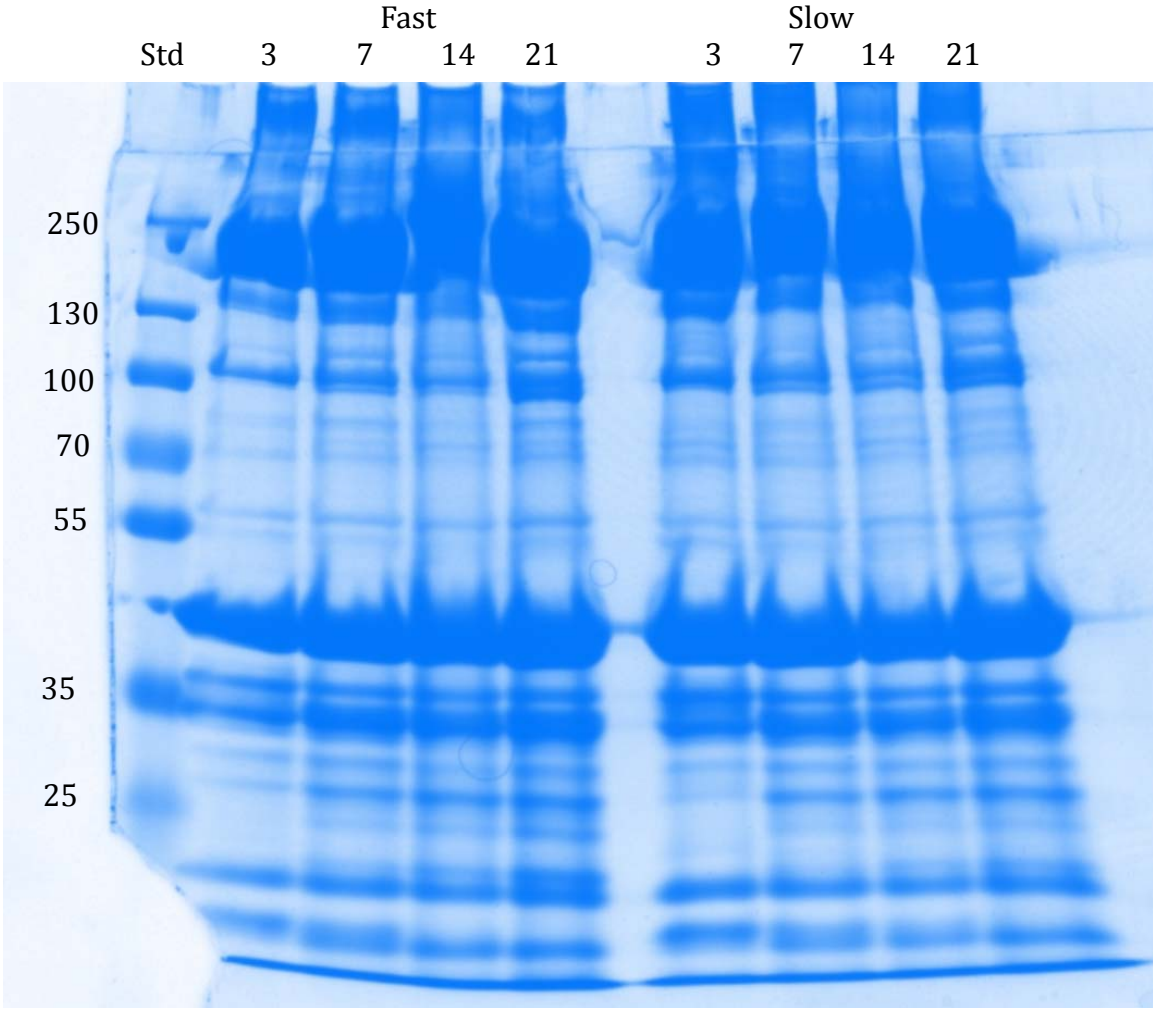

Figure 4. Ten percent SDS-PAGE gel of isolated myofibrils from muscle of the fastest growth rate, and the slowest growth rate from the 30 steers. The muscle was aged 3, 7, 14, and 21 days, at 4°C before MF Isolation, corresponding with the numbers along the top, along with the molecular weight standard.

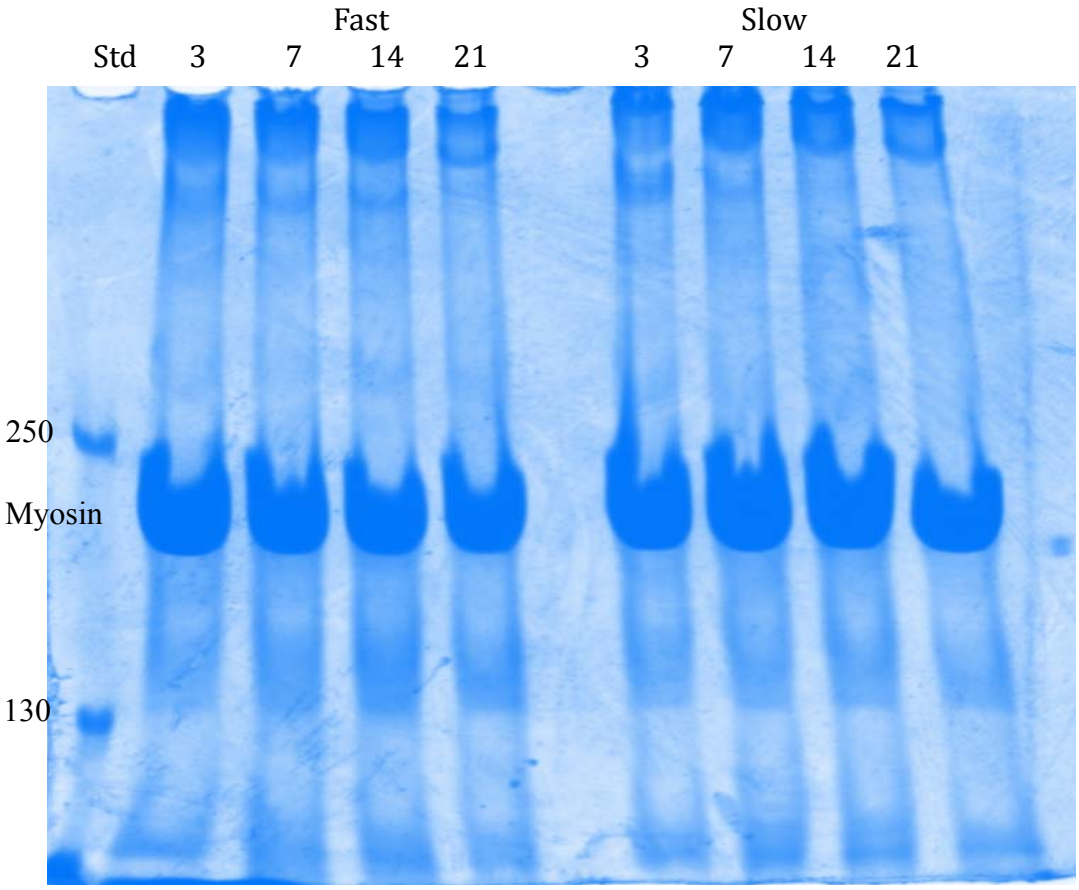

Figure 5. Five percent SDS-PAGE gel of isolated myofibrils from muscle of the fastest growth rate, and the slowest growth rate from the 30 steers. The muscle was aged 3, 7, 14, and 21 days, at 4°C, before MF Isolation, corresponding with the numbers along the top, along with the molecular weight standard.

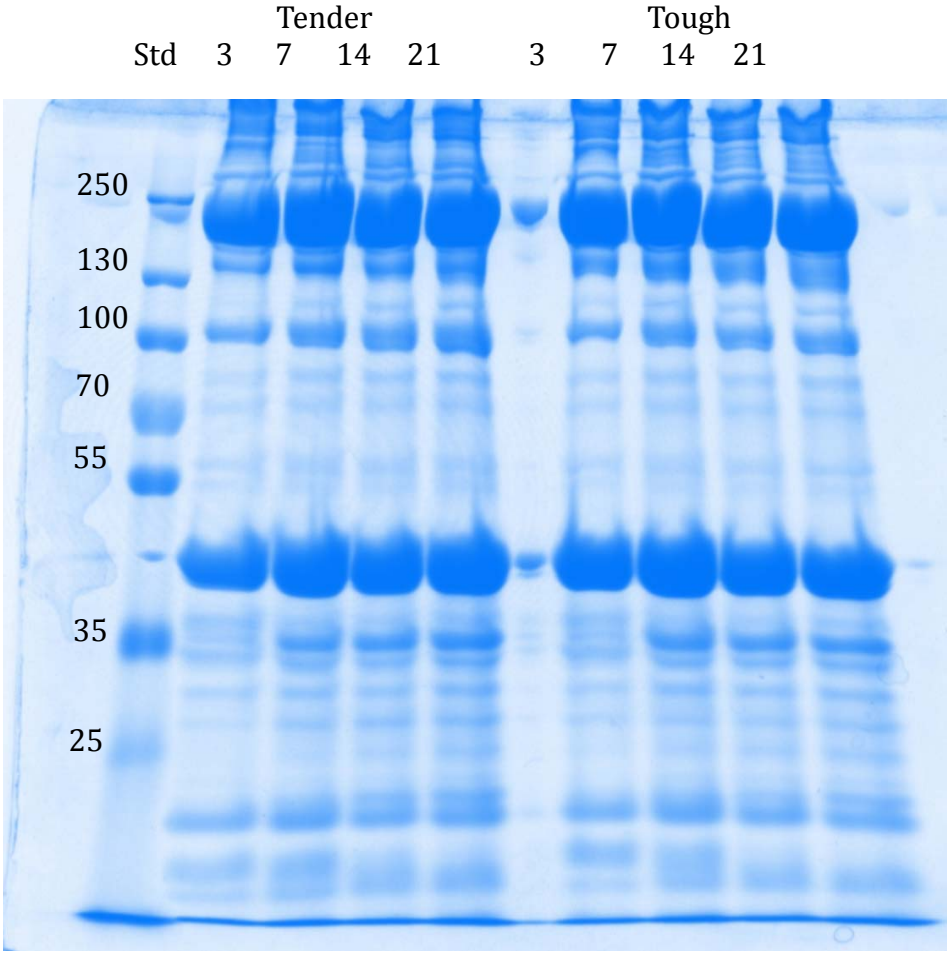

Figure 6. Ten percent SDS-PAGE gel of isolated myofibrils from muscle of the most tender and the least tender from the 30 steers. The muscle was aged 3, 7, 14, and 21 days, at 4°C, before MF Isolation, corresponding with the numbers along the top, along with the molecular weight standard.

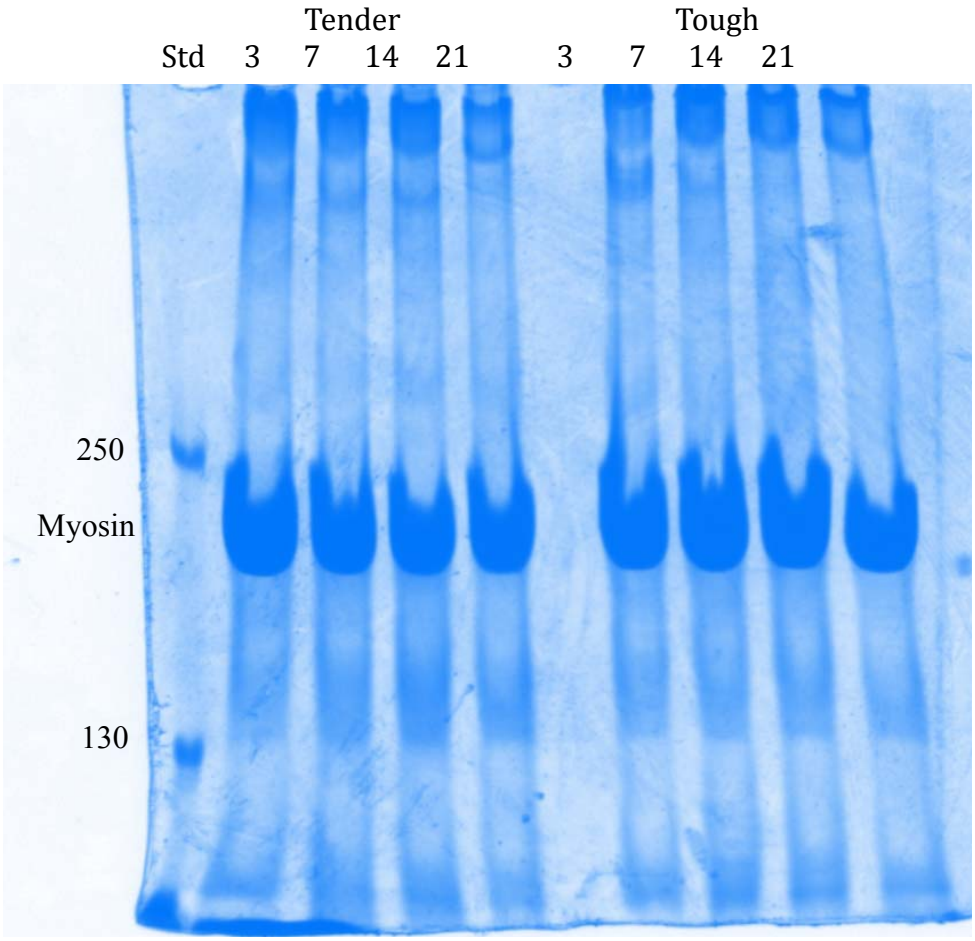

Figure 7. Five percent SDS-PAGE gel of isolated myofibrils from muscle of the most tender and the least tender from the 30 steers. The muscle was aged 3, 7, 14, and 21 days, at 4°C, before MF Isolation, corresponding with the numbers along the top, along with the molecular weight standard.

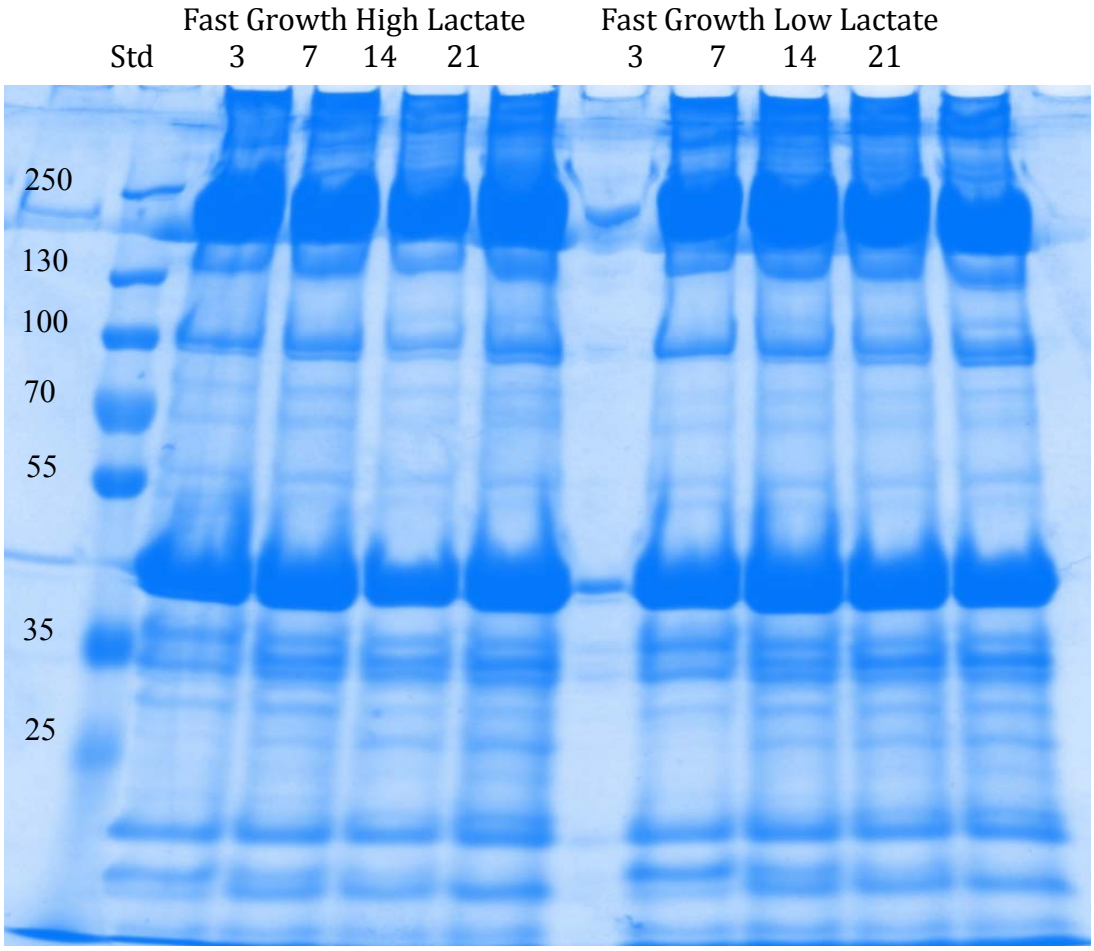

Figure 8. Ten percent SDS-PAGE gel of isolated myofibrils from muscle of the fastest growth rate paired with the highest average lactate score and the fastest growth rate paired with the lowest average lactate score from the 30 steers. The muscle was aged 3, 7, 14, and 21 days, at 4°C, before MF Isolation, corresponding with the numbers along the top, along with the molecular weight standard.

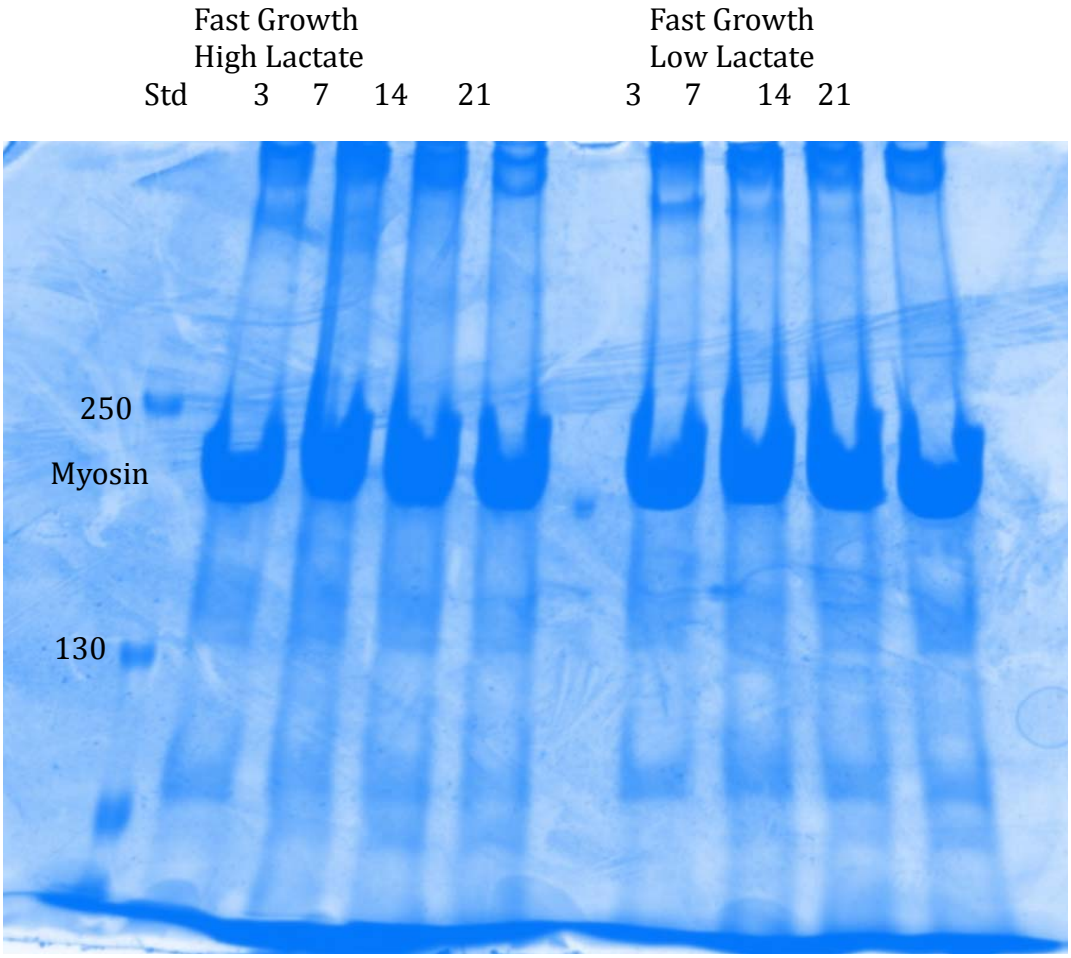

Figure 9. Five percent SDS-PAGE gel of isolated myofibrils from muscle of the fastest growth rate paired with the highest average lactate score and the fastest growth rate paired with the lowest average lactate score from the 30 steers. The muscle was aged 3, 7, 14, and 21 days, corresponding with the numbers along the top, along with the molecular weight standard.

When evaluating 10% gels, there was an appearance of the 30kDa band with increased postmortem ageing time, agreeing with Negishi et al, (1996), MacBride and Parrish, (1977), Olson et al, (1977), and Parrish et al, (1981) indicating the appearance of a 30 kDa band on SDS-PAGE after postmortem ageing. However, the gel in Figure 6 didn't indicate a difference in the 30kDa component as postmortem ageing time increased for myofibrils isolated from more tender muscle. This is in contrast to Negishi et al, (1996), MacBride and Parrish, (1977), Olson et al, (1977), and Parrish et al, (1981) indicating a reduction in the intensity of the Troponin T band with a concomitant increase in the 30 kDa band earlier postmortem in more tender samples. Furthermore, the data presented here disagrees with Huff-Lonergan et al, 2010; Olson et al, 1976; MacBride and Parrish, 1977; Koohmaraie, 1992; Thomson et al, 1996; Campo et al, 1999; Lamare et al, 2002 as they stated the 30 kDa component was strongly correlated to the tenderness of beef.

Unfortunately, within all of the 5% gels, there were no significant differences in the banding patterns when comparing the gels. Koohmaraie (1992), and Huff-Lonergan et al, (1996) found at early times postmortem (3 days) titin appeared as a distinct doublet, with the lower band, T2 more evident at day 3 than at day 1. However, the T2 band in the MF samples was always faint at day 0. The difference in the data could be due to not having a 24 hours sample and titin had already been degraded because the earliest samples we had were after 3 days of postmortem ageing. Huff-Lonergan et al, (1995) stated nebulin was degraded postmortem, with a significant difference observed at 3 days postmortem. In less-tender samples, the intact nebulin band was still easily detectable 3

days postmortem; however, more tender samples displayed only a trace of intact nebulin on SDS-PAGE. This indicated that nebulin generally was degraded faster in samples that were classified as more tender. Paterson and Parrish, (1987) also found very little nebulin detected in either infraspinatus or the rhomboideus muscles when each was aged 7 days postmortem. Again, the difference between our samples and those reported in the literature is that we were unable to obtain samples before 3 day postmortem. This could have resulted in our not seeing any band differences especially in the high molecular weight proteins.

5. CONCLUSIONS

The relationship between the different accepted measures of temperament and blood lactate concentration were significant but not high. The low relationship would suggest that the measures are related but other factors are contributing to differences. Furthermore, growth rate and blood lactate classifications indicated that tenderness was impacted by different physiological conditions that happen during the growing phase of animals such as muscle protease activity or metabolic processes that would affect the physiological age of an animal. Combining temperament data with tenderness data reported here indicate that if an animal is temperamental based on exit scores and lactate concentrations before harvest, this, could set the stage for a postmortem process that results in a less tender product. The impact of temperament or other form of stress could lead to variations seen in tenderness in the marketplace. Utilizing a simple objective measure of temperament using a lactate meter could possibly lead to decreasing variations in temperament within a producers herd and could lead to less variation in the tenderness a consumer will encounter. Currently, the most common measurement of temperament is subjective chute scores. Our data would indicate the chute score is not as good at predicting altered tenderness as exit speed. However, the hardware required to measure exit speeds is expensive and would, therefore, not be used in a normal production setting. The significant correlation between exit speed and blood lactate suggests that this easy objective measurement could be used to sort animals into categories. However, more research is needed to determine what the optimum blood lactate concentration would be as the steaks from steers with high and low lactate

concentration were more tender than were the steaks from steers with medium lactate concentrations. Adding an objective measurement of temperament to the producers' resources for selection could result in more consistent tenderness of steaks in the marketplace culminating in a greater consumer satisfaction.

LITERATURE CITED

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

Allen, R.E., Masak, K.C., McAllister, P.K., and Merkel, R,A. 1983.
Effect of growth hormone, testosterone and serum concentration on actin synthesis in cultured satellite cells. Journal of Animal Science 56: 833-837.

Allen, R.E., Merkel, R.A., and Young, R.B. 1979. Cellular aspects of muscle growth: myogenic cell proliferation. Journal of Animal Science 49: 1979.

Apple, J.K., Dikeman, M.E., Minton, J.E., McMurphy, R.M., Fedde, M.R., Leith, D.E., and Unruh, J.A. 1995. Effects of restraint and isolation stress and epidural blockade on endocrine and blood metabolite status, muscle glycogen metabolism, and incidence of dark-cutting longissimus muscle of sheep. Journal of Animal Science 73: 2295-2307.

Behrends, S.M., Miller, R.K., Rouquette, F.M., Randel, R.D., Warrington, B.G., Forbes, T.D.A., Welsh, T.H., Lippke, H., Behrends, J.M., G.E. Carstens, and Holloway J.R. 2009. Relationship of temperament, growth, carcass characteristics and tenderness in beef steers. Meat Science 81: 433-438.

Beltran, J.A., Jaime, I., Santolaria, P., Sanudo, C., Alberti, P., and Roncales P. 1997. Effect of stress-induced high post-mortem pH on protease activity and tenderness of beef. Meat Science 45: 201-207.

Bochtler, M., Ditzel, L., Groll, M., Hartmann, C., and R. Huber. 1999. Annual Review Biophysiology Biomololecular Structure 28: 295-317.

Boleman, J., Boleman, S.L., Miller, R.K., J.F., Taylor, Cross, H.R., Wheeler, T.L., Koohmaraie, M., Shackelford, S.D., Miller, M.F., West, R.L., Johnson, D.D., and Savell, J.W. 1997. Consumer Evaluation of Beef of Known Categories of Tenderness. Journal of Animal Science 75: 1521-1524.

Boles, J.A., Boss, D.L., Neary, K.I., Davis, K.C., and Tess, M.W. 2009. Growth implants reduced tenderness of steaks from steers and heifers with different genetic potentials for growth and marbling. Journal of Animal Science 87: 269-274.

Bouton, P.E., Ford, A.L., Harris, P.V., and Ratcliff, D. 1975. Objective-subjective assessment of meat tenderness. Journal of Texture Studies 6: 315-328.

Brady, P.L. and Hunecke, M.E. 1985. Correlations of Sensory and Instrumental Evaluations of Roast Beef Texture. Journal of Food Science 50: 300-303.

Burrow, H.M., Seifert, G.M., and Corbet, N.J. 1988. A new technique for measuring temperament in cattle. Proceedings Australian Society of Animal Science 17: 154-157.

Butler, G., Poste, L.M., Mackie, D.A., and Jones, A. 1996. Time-intensity as a tool for the measurement of meat tenderness. Food Quality and Preference 7: 193-204.

Café, L.M., McIntyre, B.L., Robinson, D.L., Geesink, G.H., Barendse, W., Pethick, D.W., Thompson, J.M., and Greenwood, P.L. 2010. Production and processing studies on calpain-system gene markers for tenderness in Brahman cattle: 2. Objective meat quality. Journal of Animal Science 88: 3059-3069.

Campo, M.M., Sanudo, C., Panea, B., Alberti, P., and Santolaria, P. 1999. Breed type and ageing time effects on sensory characteristics of beef strip loin steaks. Meat Science 51: 383-390.

Clark, K.A., McElhinny, A.S., Beckerle, M.C., and C.C. Gregorio. 2002. Striated muscle cytoarchitecture: an intricate web of form and function. Annual Review of Cell and Developmental Biology 18: 637-706.

Crookshank, H.R., Elissalde, M.H., White, R.G., Clanton, D.C., and Smalley, H.E. 1979. Effect of transportation and handling of calves upon blood serum composition. Journal of Animal Science 48: 430-435.

Culbert, J., and Wells, J.W. 1975. Aspects of adrenal function in the domestic fowl. Journal of Endocrinology 64: 363-376.

Culler, R.D., Parrish, F.C., Smith, G.C., and Cross, H.R. 1978. Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. Journal of Food Science 43: 177-1180.

Curley, K.O., Paschal, J.C., Welsh, T.H., and Randel, R.D. 2006. Technical note: exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. Journal of Animal Science 84: 3100-3103.

Delius, J.D., Craig, B., and Chaudoir, C. 1976. Adrenocorticotropic hormone, glucose and displacement activities in pigeons. Z Tierpsychol 40: 183-193.

Devine, C.E., Lowe, T.E., Wells, R.W., Edwards, N.J., Hocking Edwards, J.E., Starbuck, T.J., and Speck, P.A. 2006. Pre-slaughter stress arising from on-farm handling and its interactions with electrical stimulation on tenderness of lambs. Meat Science 73: 304-312.

Dikeman, M.E. 1987. Fat reduction in animals and the effects on palatability and consumer acceptance of meat products. Proceedings of Reciprocal Meat Conference 40:93.

Dransfield, E., Jones, R.C., and MacFie, H.J. 1981. Quantifying changes in tenderness during storage of beef. Meat Science 5: 131-137.

Duizer, L.M., Gullett, E.A., and Findlay, C.J. 1993. Time-intensity methodology for beef tenderness perceptions. Journal of Food Science 58: 943-947.

Eilers, J.D., Tatum, J.D., Morgan, and Smith, C.J. 1996. Modification of early-postmortem muscle pH and use of postmortem ageing to improve beef tenderness. Journal of Animal Science 74: 790-798.

Ferguson, D.M., Jiang, S.T., Hearnshaw, H., Rymill, S.R., and Thompson, J.M. 2000. Effect of electrical stimulation on protease activity and tenderness of M.longissimus from cattle with different proportions of Bos indicus content. Meat Science 55: 265-272.

Ferguson, D.M., Johnston, D., Burrow, H., and A. Reverter. 2006. Relationships between temperament, feedlot performance and beef quality. Australian Beef- The Leader! The impact of science on the beef industry. Cooperative Research Centre for Beef Genetic Technologies, Armidale, New South Wales, Australia.

Fishell, V.K., Aberle, E.D., Judge, M.D., and Perry, T.W. 1985. Palatability and muscle properties of beef as influenced by preslaughter growth rate. Journal of Animal Science 61: 151-157.

Freeman, B.M., and Manning, A.C. 1982. An evaluation of adrenal mass and adrenal cholesterol as measures of adrenal activity. British Poultry Science 23: 257-262.

Freeman, B.M. 1983. The adrenal glands. Biochemistry and Physiology of the Domestic Fowl. B.M. Freeman, ed. Academic Press, New York, pp. 191-209.

Fordyce, G., Dodt, R.M. and Wythes, J.R. 1988. Cattle temperaments in extensive beef herds in northern Queensland 1. Factors affecting temperament. Australian Journal of Experimental Agriculture 28: 683-687.

Goll, D.E., Young, R.B. and Stromer, M.H. 1974. Separation of subcellular organelles by differential and density gradient centrifugation. Proceedings of Reciprocal Meats Conference 27:250-297.

Goll, D.E., Thompson, V.F., Taylor, R.G., and Ouali, A. 1998. The calpain system and skeletal muscle growth. Canadian Journal of Animal Science 78: 503-512.

Goll, D., Thompson, V., Taylor, R.G., and Christiansen, J.A. 1992. Role of the calpain system in muscle growth. Biochimie 74: 225-237.

Goll, D., Otsuka, Y., Nagainis, A., Shannon, J., Sathe, S., and Muguruma, M. 1983. Role of muscle proteinases in maintenance of muscle integrity and mass. Journal of Food Biochemistry 7: 137-177.

Graafhuis, A.E., Honikel, K.O., Devine, C.E. and Chrystall, M.M. 1991. Tenderness of different muscles cooked to different temperatures and assessed by different methods. International Congress of Meat Science and Technology 3: 365.

Grandin, T. 1993. Behavioral agitation during handling of cattle is persistent over time. Applied Animal Behaviour Science 36: 1-9.

Greaser, M. 1991. An overview of the muscle cell cytoskeleton. Proceedings of the Reciprocal Meat Conference 44: 1-5.

Goldstein, M.A., Michael, L.H., Schroeter, J.P., and Sass, R.L. 1986. The Z-band lattice in skeletal muscle before, during and after tetanic contraction. Journal of Muscle Research and Cell Motility 7: 527-536.

Hawke, T. J., and Garry, D.J. 2001. Myogenic satellite cells: physiology to molecular biology. Journal of Applied Physiology 91: 534-551.

Holmes, J.H.G., Ashmore, C.R., and Robinson, D.W. 1973. Effects of stress on cattle with hereditary muscular hypertrophy. Journal of Animal Science 36: 684-694.

Hopkins, D.L., Littlefield, P.J., and Thompson, J.M. 2000. A research note on factors affecting the determination of myofibrillar fragmentation. Meat Science 56: 19-22.

Huang, J., and Forsberg, N.E. 1998. Role of calpain in skeletal-muscle protein degradation. Proceedings National Academic Science, USA 95: 12100-12105.

Huff-Lonergan, E., Mitsuhashi, T., Beekman, D.D., Parrish, F.C., Olson, D.G., and Robson, R.M. 1996. Proteolysis of specific muscle structural proteins by mu-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. Journal of Animal Science 73: 993-1008.

Huff-Lonergan, E., Mitsuhashi, T., Parrish, F.C., and Robson, R.M. 1996. sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting comparisons of purified myofibrils and whole muscle preparations for evaluating titin and nebulin in postmortem bovine muscle. Journal of Animal Science 74: 779-785.

Huff-Lonergan, E., Parrish, F.C., and Robson, R.M. 1995. Effects of postmortem ageing time animal age, and sex on degradation of titin and nebulin in bovine longissimus muscle. Journal of Animal Science 73: 1064-1073.

Huff-Lonergan, E., Zhang, W., and Lonergan, S.M. 2010. Biochemistry of postmortem muscle- Lessons on mechanisms of meat tenderization. Meat Science 86: 184-195.

Hwan, S.F., and Bandman, E. 1989. Studies of desmin and α-actinin degradation in bovine semitendinosus muscle. Journal of Food Science 54:1426-1430.

Hwang, I.H., and Thompson, J.M. 2001. The effect of time and type of electrical stimulation on the calpain system and meat tenderness in beef longissimus dorsi muscle. Meat Science 58: 135-144.

Immonen, K., Ruusunen, M., and Puolanne, E. 2000. Some effects of residual glycogen concentration on the physical and sensory quality of normal pH beef. Meat Science 55: 33-38.

Jeremiah, L.E., Tong, A.K., and Gibson, LL. 1991. The usefulness of muscle color and ph for segregating beef carcasses into tenderness groups. Meat Science 30: 97-114.

Karlsson, A, Enfalt, A.C., Essen-Gustavsson, B.E., Lundstrom, K., Rydhmer, L, and Stern, S. 1993. Muscle histochemical and biochemical properties in relation to meat quality during selection for increased lean tissue growth rate in pigs. Journal of Animal Science 71: 930-938.

Karlsson, A., Enfalt, A.C., Essen-Gustavsson, B.E., King, D.A., Schuehle, C.E., Randel, R.D., Welsh, T.H., Oliphint, R.A., Baird, B.E., Curley, K.O., Vann, R.C., Hale, D.S., and Savell, J.W. 2006. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. Meat Science 74: 546-556.

Koohmaraie, M. 1992. The role of Ca^{2+} dependent proteases (calpains) in postmortem proteolysis and meat tenderness. Biochimie 74: 239.

Koohmaraie, M., Kent, M.P., Shackelford, S.D., Veiseth, E., and Wheeler, T.L. 2002. Meat tenderness and muscle growth: is there any relationship? Meat Science 62: 345-352.

Lamare, M., Taylor, R.G., Farout, L., Briand, Y., and Briand, M. 2002. Changes in proteasome activity during postmortem ageing of bovine muscle. Meat Science 61: 199-204.

Luther, P., and Squire, J. 1978. Three-dimensional structure of the vertebrate muscle M-region. Journal of Molecular Biology 125: 313-324.

MacBride, M.A., and Parrish, F.C. 1977. The 30,000-Dalton Component of Tender bovine longissimus muscle. Journal of Food Science 42: 1627-1629.

Marsh, B.B. 1977. Symposium The basis of quality in muscle foods, the basis of tenderness in muscle foods. Journal of Food Science 42: 295-297.

Monson, F., Sanudo, C., and Sierra, I. 2004. Influence of cattle breed and ageing time on textural meat quality. Meat Science 68: 595-602.

Morgan, J.B., Savell, J.W., Hale, D.S., Miller, R.K., Griffin, D.B., Cross, H.R., and Shackelford, S.D. 1991. National Beef Tenderness Survey. Journal of Animal Science 69: 3274-3283.

Nishimura, T., Liu, A., Hattori, A., and Takahashi, K. 1998. Changes in mechanical strength of intramuscular connective tissue during postmortem ageing of beef. Journal of Animal Science 76: 528-532.

Oddy, V.H., Harper, G.S., Greenwood, P.L., and McDonagh, M.B. 2001. Nutritional and developmental effects on the intrinsic properties of muscles as they relate to the eating quality of beef. Australian Journal of Experimental Agriculture 41: 921-942.

O'Halloran, G.R., Troy, D.J., and Buckley, D.J. 1994. The influence of ultimate pH on the tenderization of meat. Proceeding of the International Congress of Meat Science and Technology 2: 432-435.

O'Halloran, G.R., Troy, D.J., and Buckley, D.J. 1997a. The relationship between early post-mortem pH and the tenderization of beef muscles. Meat Science 45: 239-251.

O'Halloran, G.R., Troy, D.J., Buckley, D.J., and Reville, W.J. 1997b. The role of endogenous proteases in the tenderization of fast glycolysing muscle. Meat Science 47: 187-210.

Olson, D.G., Parrish, F.C., Dayton, W.R., and Goll, D.E. 1977. Effect of postmortem storage and calcium activated factor on the myofibrillar proteins of bovine skeletal muscle. Journal of Food Science 42: 117-124.

Olson, D., Parrish, F., and Stromer, D.B. 1976. Myofibrillar fragmentation and shear resistance of three bovine muscles during postmortem storage. Journal of Food Science 41: 1036-1041.

Partida, J.A., Olleta, J.L., Campo, M.M., Sanudo, C., and Maria, G.A. 2007. Effect of social dominance on the meat quality of young Friesian bulls. Meat Science 76: 266-273.

Pearson, A.M., Wolzak, A.M., and Gray, J.I. 1983. Possible role of muscle proteins in flavor and tenderness of meat. Journal of Biochemistry 7: 189-210.

Perry, D., Thompson, J.M., Hwang, I.H., Butchers, A. and Egan, A.F. 2001. Relationship between objective measurements and taste panel assessment of beef quality. Australian Journal of Experimental Agriculture 41: 981-989.

Platter, W.L., Tatum, J.D., Belk, K.E., Chapman, P.L., Scanga, J.A., and Smith, G.C. 2003. Relationships of consumer sensory ratings, marbling score, and shear force value to consumer acceptance of beef strip loin steaks. Journal of Animal Science 81: 2741- 2750.

Purchas, R.W. 1990. An assessment of the role of pH differences in determining the relative tenderness of meat from bulls and steers. Meat Science 27: 129-140.

Purchas, R.W. and Aungsupakom, R. 1993. Further investigations into the relationship between ultimate pH and tenderness for beef samples from bulls and steers. Meat Science 34: 163-178.

Purchas, R.W., Burnham, D.L., and Morris, S.T. 2002. Effects of growth potential and growth path on tenderness of beef longissimus muscle from bulls and steers. Journal of Animal Science 80: 3211-3221.

Purchas, R.W., Yan, X., and Hartley, D.G. 1999. The influence of a period of ageing on the relationship between ultimate pH and shear values of beef m. longissius thoracis. Meat Science 51: 135-141.

Sanz, M.C., Verde, M.T., Saez, T., and Sanudo, C. 1996. Effect of breed on the muscle glycogen content and dark cutting incidence in stressed young bulls. Meat Science 43: 37-42.

Sawada, II., Ishikawa, II. and Yamada, E. 1978. High resolution scanning electron microscopy of frog Sartorius muscle. Tissue Cell 10: 179-190.

Shackelford, S.D., Koohmaraie, M., and Savell, J.W. 1994. Evaluation of Longissimus dorsi muscle pH at three hours post mortem as a predictor of beef tenderness. Meat Science 37: 195-204.

Shackelford, S.D., Wheeler, T.L., and Koohmaraie, M. 1995. Relationship between shear force and trained sensory panel tenderness ratings of 10 major muscles from *bos indicus* and *bos taurus* cattle. Journal of Animal Science 73: 3333-3340.

Silva, J.A., Patarata, L., and Martins, C. 1999. Influence of ultimate pH on bovine meat tenderness during ageing. Meat Science 52: 453-459.

Solomon, V., and Goldberg, A.L. 1996. Importance of the ATP-ubiquitin-proteasome pathway in the degradation of soluble and myofibrillar proteins in rabbit muscle extracts. The Journal of Biological Chemistry 271: 26690-26697.

Steen, D., Claeys, E., Uytterhaegen, L., DeSmet, S., and Demeyer, A. 1997. Early post-mortem conditions and the calpain/calpastatin system in relation to tenderness of double-muscled beef. Meat Science 45: 307-319.

Takahashi, K. 1996. Structural weakening of skeletal muscle tissue during post-mortem ageing of meat: the non-enzymatic mechanism of meat tenderization. Meat Science 43: S67-S80.

Tatum, J.D., Belk, K.E., George, M.H., and Smith, G.C. 1999. Identification of Quality management practices to reduce the incidence of retail beef tenderness problems: Development and evaluation of a prototype quality system to produce tender beef. Journal of Animal Science 77: 2112-2118.

Tarrant, P.V., Kenny, F.J., Harrington, D., and Murphy, M. 1992. Long distance transportation of steers to slaughter: effect of stocking density on physiology, behavior and carcass quality. Livestock Production Science 30: 223-238.

Tarrant, P.V., and Mothersill, C. 1977. Glycolysis and associated changes in beef carcasses. Journal of Science Food and Agriculture 28: 739-749.

Thomson, B.C., Dobbie, P.M., Singh, K., and Speck, P.A. 1996. Post-mortem kinetics of meat tenderness and the components of the calpain system in bull skeletal muscle. Meat Science 44: 151-157.

Veiseth, E., Shackelford, S.D., Wheeler, T.L., and Koohmaraie, A. 2001. Effect of postmortem storage on m-calpain and μ-calpain in ovine skeletal muscle. Journal of Animal Science 79: 1502-1508.

Villarroel, M., Maria, G.A., Sanudo, C., Olleta, J.L., and Gebresenbet, G. 2003. Effect of transport time on sensorial aspects of beef meat quality. Meat Science 63: 353-357.

Voisinet, B.D., Grandin, T., O'Connor, S.F., Tatum, J.D., and Deesing, M.J. 1997. Bos indicus-cross feedlot cattle with excitable temperaments have tougher meat and a higher incidence of borderline dark cutters. Meat Science 4: 367-377.

Wang, K.M., and Tu, A. 1979. Titin: major myofibrillar components of striated muscle. Proceedings of National Academic Science. USA 76: 3698-3702.

Watanabe A., and Devine, C. 1996. Effect of meat ultimate pH on rate of titin and nebulin degradation. Meat Science 42: 407-413.

Wells, J.W., and P.L. Wight. 1971. The adrenal glands. In: Physiology and Biochemistry of the Domestic Foul. D.J. Bell, and B.M. Freeman, eds. Academic Press, London, pp. 489-520.

Wolcott, M.L., Johnston, D.J., Barwick, S.A., Iker, C.L., Thompson, J.M., and Burrow, M.E. 2009. Genetics of meat quality and carcass traits and the impact of tenderstretching in two tropical beef genotypes. Animal Production Science 49: 383-398.

Woodhead, J.L., and Lowey, S. 1983. An in Vitro study of the interactions of skeletal muscle M-protein and creatine kinase with myosin and its subfragments. Journal of Molecular Biology 168: 831-846.

Wulf, D.M., Morgan, J.B., Tatum, J.D., and Smith, G.C. 1996. Effects of animal age, marbling score, calpastatin activity, subprimal cut, calcium injection, and degree of doneness on the palatability of steaks from limousin steers. Journal of Animal Science 74: 569-576.

Yamaguchi, M., Izuminmoto, M., Robson, R.M., and Stromer, M.H. 1985. Fine structure of wide and narrow vertebrate muscle Z-lines. A proposed model and computer simulation of Z-line architecture. Journal of Molecular Biology 184: 621-644.

Yu, L.P., and Lee, Y.B. 1986. Effects of postmortem pH and temperature on bovine muscle structure and meat tenderness. Journal of Food Science 51_ 774- 780.

Zeece, M.G., Robson, R.M., Lusby, M.L., and Parrish, F.C. 1986. Effect of calcium activated protease (CAF) on bovine myofibrils under different conditions of pH and temperature. Journal of Food Science 51: 797-803.

Zhang, M., and Mittal, G.C. 1993. Measuring tenderness of meat products by Warner Bratzler shear press. Journal of Food Processing and Preservation 17: 351-367.