

GENETIC MARKERS, CATTLE DISPOSITION, AND STRESS RELATIVE TO
VARIATIONS IN BEEF TENDERNESS

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Genetic Markers, Cattle Disposition, and Stress Relative to Variations in Beef Tenderness

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ABSTRACT

The objective of this research was to evaluate the efficacy of genetic markers, disposition, and animal stress on variations in beef tenderness. Warner-Bratzler shear force (WBSF) values on 570 mixed breed heifers and steers were used to determine estimates of genetic selection. Cattle used for this analysis were marketed from 2008 to 2011, and included five different feedlot based research projects at the Carrington Research Extension Center (Carrington, ND). Tissue samples were collected for IGENITY[®] (Merial Limited, Duluth, GA) analysis. Results included both selection indices and molecular breeding values for hot carcass weight, ribeye area, yield grade, fat thickness, percent choice, marbling, tenderness, docility, heifer pregnancy rate, maternal calving ease, and stayability. These genetic based parameters were compared with actual carcass values and measurements of temperament including exit velocity, chute score, and capture score. Genetic marker assisted selection may offer a more effective means of improving cattle management strategies and product quality; however there is progress to be made on the accuracy of such predictions. In the second project, the effect of temperament and slaughter method on Minolta color scores and tenderness was evaluated. Measurements of temperament were obtained prior to slaughter on Angus x Peidmontese crossbred heifers. Heifers were slaughtered on two consecutive Mondays using either Kosher or captive-bolt slaughter methods. At approximately 24 h post-mortem, carcass measurements and marbling scores were obtained. *Longissimus thoracis* (LT) samples were collected and aged 14 d prior to Minolta color score and WBSF measurements. Chute score, capture score, and vocalization scores significantly correlated ($P < 0.03$) with blood lactate concentration. The LT from Kosher slaughtered heifers had significantly higher ($P < 0.01$) L*, a*, b* and WBSF values than that of captive bolt stunned heifers. The LT from captive bolt stunned heifers had

significantly higher ($P = 0.04$) marbling, and a tendency ($P = 0.08$) for increased cook loss compared with that from Kosher slaughtered heifers. These data indicate that chute behavior is significantly correlated to measurements of blood lactate and suggests that the Kosher slaughter method may negatively affect meat quality parameters compared with the captive-bolt stunning method.

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CHAPTER I. INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Tenderness is the most studied palatability trait of cooked meat, and one of the most important factors influencing consumer acceptability of beef (Beermann, 2009). The Beef Customer Satisfaction Study (Neely et al., 1998; 1999) also showed that tenderness can be a major contributing factor to the consumer's perception of taste. While the average consumer is concerned with price per serving, it is the eating experience that will keep the beef consumers coming back for more. Due to the fact there are a myriad of factors that affect tenderness, it is important to maintain a persistent focus on all traits to ensure beef palatability and a high level of consumer acceptability. Some of these influential factors on tenderness include genetics, time on feed, nutrition, growth promotants, age, stress, chilling rate, and aging of the product (Tatum et al., 2007). Marbling has also been shown to have a small but positive influence on tenderness, along with influencing other palatability traits such as juiciness and flavor (Wheeler et al., 1994).

With such a variety of genetic and environmental traits influencing tenderness, it has been a challenge for producers and processors alike to hone in on improving beef tenderness. While it has been a challenge, progress has been made. Voges et al. (2007) reported in the National Beef Tenderness Survey that Warner- Bratzler shear force values had improved from previous data collected in 1991 and 1998. The authors credited increased aging times, longer and slower carcass chilling rates and an improved focus on beef tenderness programs. While progress has been made, much interest remains, as there is a financial incentive involved with improving tenderness, due to the potential higher premium consumers are willing to pay for a guaranteed tender product (Boleman et al., 1997). On the same hand, consumers are not willing to pay for an unsatisfactory product, and therefore one negative experience can negate many

years of improvement in beef palatability. So while we focus on using tenderness to improve profitability, we shall also consider the research with due diligence to insure acceptability of current and future beef consumers.

While the consumer will judge the product by eating characteristics, it is important to first supply a product they are willing to purchase based on appearance. Overall product quality and retail case appearance, affected by lean and fat color, water-holding capacity, shelf life, and the lean: fat ratio, will all influence purchasing decisions. Quality is a term that is used a lot, but often difficult to define. Troy (1999) describes quality as “a measure of traits that are sought and valued by the consumer.” Hoffman (1990) went into more detail and defined quality as the “sum of all quality factors of meat in terms of the sensoric, nutritive, hygienic, toxicological and technical properties.” It is my belief that we must be cognizant of these other parameters besides the phenotypic and sensoric properties that we typically evaluate in order to move forward in our understanding of beef quality.

Due to the perceived need for continuous improvement in retail case uniformity, much technology has surfaced to help producers in selecting the cattle that best fit their operation, and the needs of the consumer. Genetic testing is a technology that has surfaced in the commercial market within the last 10 years to help producers make selection decisions effecting economically significant traits. While genetic testing is still a few years away from characterizing retail case performance, other quality predictions such as quality and yield grade, tenderness, and ribeye area size can be used to improve uniformity. Similarly, these technologies can be applied to the feedlot as well, allowing for prediction of feed and growth efficiency, expected growth rate, marbling potential, and even estimating the docility of that animal.

The beef industry continues to shift and adapt to ever changing issues facing producers. From the selection of much leaner genetics in the 1970's and 1980's to the recent focus on selecting cattle that are more efficient and potentially more profitable to the producer. Whether we are dealing with droughts or increasing feed costs as a result of competition in the international markets and our national ethanol policy, cost of production changes. Fortunately, U.S. and international beef demand continues to rise, with historically high carcass prices offsetting the historically high cost of production. The U.S. beef industry is strong, and with a continued focus to improving production practices, animal welfare, and meat quality, consumers expect the quality of their beef eating experience to improve as well. Research institutions and commodity associations are important service groups assisting producers in understanding and utilizing new technologies to both improve their product today, but also to insure their survivability in the market place tomorrow.

Factors Affecting Tenderness

With beef being a higher priced protein at the dinner table, a positive eating experience and consumer acceptability are traits that help drive the demand for beef. In 1998, the National Beef Tenderness Survey (Brooks et al., 2000) showed much improvement in retail beef tenderness compared to the 1990 National Beef Tenderness Survey.

Factors that are known to influence beef tenderness include genetics, time on feed, nutrition, use of growth promoting implants, age at slaughter, stress, carcass chilling rate, state of muscle contraction, carcass aging, extent of proteolytic degradation and amount of connective tissue (Tatum, 2007). While this list is not inclusive to all known traits of influence, most research has reported these are the most influential factors when it comes to tenderness. It is important to point out however that all steps in the production and processing chain can

influence tenderness, and that overall product improvement is a responsibility of both the producer and the packing plant. For this review, the focus will be on those traits the producer has control with regard to genetic selection and feedlot management, as well as the factors under the direction of the packing plant leading up to the time of slaughter.

Influence of Genetics

Hocquette et al. (2006) described beef quality as a combination of muscle characteristics of live animals and post-mortem factors affecting the ageing process. Specifically the authors noted that genetics, nutrition, and rearing factors could influence these muscle characteristics. Without a doubt, the first aspect that livestock production producers have control when selecting beef cattle is genetics. Whether building a new herd or improving an existing one, genetic selection of superior males and females is something every producer has control over to improve the genetic potential of their herd. While finances will play a role in selection protocols and outcomes, utilizing sires and dams that better fit the objectives of the herd will be beneficial. However, selection can be much more specific than just breed and parentage, as producers can make decisions based on other phenotypic parameters such as structure and soundness, growth rate and performance, carcass merit, reproductive potential, and even docility.

From a genetic perspective, different breeds and even different genotypes within a breed can differ in expected beef quality outcomes (Hocquette et al., 2006). Research has shown much variation in the amount of connective tissue present, the content and composition of marbling, and the characterization of the muscle fibers across genotypes and breeds (Purslow, 2005). While environmental factors will further influence these traits, the predisposition of muscle composition can lead to changes in meat color, cooking losses, flavor, and tenderness (Hocquette et al., 2006). For example, meat from *Bos indicus* cattle is tougher than meat from *Bos taurus*

cattle, primarily due to the reduction in myofibrillar protein breakdown because of the presence of calcium-dependent protease inhibitors, such as calpastatin (Whipple et al, 1990). There is also evidence that later maturing beef breeds such as the Limousin have a higher protein accretion rate and less fat deposition compared to early maturing beef breeds such as the Angus (Hocquette et al., 2006). Though the selection of later maturing breeds will not greatly influence tenderness, it will negatively impact marbling, reducing the overall flavor and palatability.

Influence of Temperament and Stress

Fear is a universal emotion that motivates animals to avoid predators (Grandin, 1997). The motivation to avoid predators can lead to numerous challenges and stressful situations throughout an animal's life, negatively impacting production efficiency, meat quality, and overall profitability. Ferguson and Warner (2008) describe stress as the inevitable consequence of transferring animals from farm to slaughter. However, other researchers include extreme weather changes, poor animal handling, inadequate nutrition, and injury as stressful situations. In today's feedlot, all meat animals will experience some level of stress prior to slaughter (Ferguson and Warner, 2008), and it is the animal's response to these situations that producers should work to minimize.

Livestock producers use the word temperament as a means to explain animal behavior in the presence of a stressful situation. Webster's dictionary defines temperament as "a characteristic or habitual inclination or mode of emotional response", while Ferguson and Warner (2008) define it as the behavioral expression of the fearfulness of an animal in response to a challenging situation. Beef cattle temperament is widely variable and can have a major impact on the producer's bottom line. Cattle with poor temperament, also referred to by producers as flighty cattle, may pose more management issues such as the need for stronger and

taller working facilities, more skilled handlers, as well as more days on feed due to a decrease in efficiency (Fordyce, 1988). Necessary improvements in handling facilities and decreased animal performance can have significant economic challenges to the feedlot owner, and thus needs to be an important area of research. Also, cattle with a hyper disposition have been shown to produce tougher meat (Voisinet, 1997a) and increased amounts of bruise trim due to injuries acquired during transportation (Fordyce, 1988), all resulting in significant financial loss to the beef industry.

The Beef Checkoff reports that most cattle today are transported two to four times in their life, making travel the second most stressful event in an animal's life; second only to extreme weather (Slagle, 2007). The stress that is associated with transport, especially the transport of fat cattle to market, can also negatively impact body composition traits (Vann, 2008). While producers assume that livestock stress is a guarantee at some point in the animal's life, it is the type, duration, and intensity of these events that will have a lasting effect on end product quality (Ferguson et al., 2001). One of the most economically significant impacts transport stress has on market cattle is the decrease in intramuscular fat (marbling), potentially decreasing the quality grade and subsequent carcass value of the animal. Intramuscular fat is one of the last deposition sites in beef cattle, and is also the easiest to mobilize in times of nutritional or environmental stress (Vann, 2008). This rate of mobilization has been shown to be more severe as transportation time to market increases, resulting from the increased stress status of the animal (Vann, 2008).

The types of stress affecting livestock can be divided into two distinct categories; psychological and physical. Psychological stress can include factors such as restraint, handling and novel situations, while physical stresses include hunger, thirst, fatigue, injury and thermal

extremes (Grandin, 1997). While the stressors can vary, the impact is typically the same. In general, stress causes an increase in heart and respiration rate, body temperature, glycogen breakdown, and a decrease in protein degradation (Bass et al., 2010); all having a negative effect on the animal (Ferguson and Warner, 2008).

A stimulus of fear results in the activation of the neuroendocrine system which is comprised of two centrally integrated processes; the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis (Ferguson and Warner, 2008). The HPA axis is activated in response to external stimuli and is an important survival mechanism that allows living organisms to maintain homeostasis (King et al., 2006). Typically small stressors, those common in the production setting (i.e. human contact and handling), will elicit a stress response. This response, regulated by the autonomic nervous system, results in increased heart and respiration rate, elevated body temperature, and redistribution of blood flow to the skeletal muscle and brain. This response is mediated by the catecholamines; epinephrine and norepinephrine. Secretion of these catecholamines is where stress begins to affect metabolism, resulting in increased lipolysis, as well as glycogenolysis in the muscle and gluconeogenesis (Kuchel, 1991). Tarrant (1989) described the significance of these pathways relative to metabolism, reporting that the rate of glycogenolysis in response to epinephrine injection is approximately 185 times higher than that observed during fasting. This resulting depletion of muscle glycogen due to stress can have detrimental effects on both feedlot performance and meat quality. Decreases in feed efficiency, growth rate, and immune function in the feedlot as well as changes in carcass pH, tenderness, aging potential, color, and water-holding capacity can all be accredited to stress (Gregory, 2003).

Pre-Slaughter Stress

While stressful events in life are guaranteed, producers and processors alike look for methods of reducing or alleviating the impact of stress. Of these stressful events, none may be more critical than during the period of transfer from the farm to the processing plant (Ferguson and Warner, 2008). While much research has been focused on the ramifications of feedlot stress and environmental stress from calving to finishing, our understanding of the effect of stressful events immediately preceding slaughter is limited. Pre-slaughter stress is an area of research that has received a small amount of attention in relation to the importance it has on end product quality (Warriss, 1990). Events leading up to the arrival of livestock at the processing plant present new and challenging situations for livestock. These stimuli include increased human contact and handling, transport, new environments, lack of access to food and water, climatic changes, as well as changes in group dynamics from changing of pen size and pen mates (Ferguson and Warner, 2008). Other aspects of change that occur at the packing plant include smells, sights, and sounds that are unfamiliar to the animal. To many cattle, entering the v-belt restrainer and having their head restrained prior to stunning can be a period of extreme stress. Lastly, and a topic that will be discussed in more detail later, is differences in slaughter technique, specifically deviations from the use of captive bolt stunning. How these practices are applied may also have a negative impact on end product quality.

Byrd et al. (1989) reported that physical stress can also lead to changes in sarcoplasmic reticulum function, altering calcium transport in the muscle. These findings would suggest that stress may also be altering post-mortem glycolysis and calpain mediated proteolysis through the above mechanism. Conversely, data by Magolski (2009) showed that while feedlot temperament did affect tenderness, these changes were not mediated by changes in postmortem proteolysis.

With regard to muscle ultrastructure, the authors hypothesized that the observed relationship between temperament and tenderness was influenced by connective tissue content and/or sarcomere length.

Other Factors

Achieving a high quality beef product is like constructing the perfect wooden spoke wheel. Every spoke must work in unison and hold their weight, because if one is lacking or missing, the end point goal becomes tougher to reach. While much effort has been spent discussing how temperament and disposition, genetics, and pre-slaughter stress influences tenderness, other factors such as nutrition, use of growth promoting implants, age, lairage time and weather also play an important role in producing a desirable product (Ferguson and Warner, 2008). Continuing with the wooden wheel analogy, there are countless spokes playing a role in tenderness, and it is important to understand as many of those factors as possible. While many would suggest that genetics is the center spoke and the foundation of future progress, the best genetics cannot excel without optimum conditions, and it is up to the producer and processor to give animals the best chance of excelling from the time that animal comes through the farm gate to the time it reaches the dinner plate.

Measuring the Influence of Genetics

The profiling of the beef genome has opened up a multitude of paths to improving beef quality. Having the ability to identify specific polymorphisms in key genes that play a role in tenderness and beef quality will make available to producers tools to aid in herd selection and improvement. Through these isolated single nucleotide polymorphisms, new molecular indicators can be developed to aid producers in the selection of cattle with improved tenderness and palatability (Hocquette et al., 2006). For tenderness, genetic variations of the calpain 1 gene,

calpastatin, and lysyl oxidase have been reported to influence phenotypic variation, and therefore have become a focus for marker assisted selection (Page et al., 2002; Barendse, 2002). Through the GeMQual project funded by the European Union, about 500 candidate genes have been categorized as having an effect on muscle development, composition, metabolism, or ageing, and therefore these genes have been the focus of understanding meat quality through physiological function (Hocquette et al., 2006). Through these genetic discoveries, it is now possible to develop DNA tests to improve beef quality by genetic selection as well as identify molecular markers that can advance or detract from beef quality, aiding in the prediction of attributes that will ultimately improve beef quality.

Genetic Testing

The ability to characterize beef cattle on genetic merit and potential has long been the result of expected progeny differential (EPD) utilization and the tracking of progeny through the production and finishing phase. As technology evolved through the sequencing of the bovine genome, DNA information now has the potential to create added value to the beef industry (Van Eenennaam and Drake, 2012). This added value can potentially be realized on different scales by all sectors of the beef industry, including seed-stock producers, commercial producers, feedlots, and processors. Across sectors, different traits are routinely of focus, thus presenting an opportunity to develop different selection parameters for different goals across the different segments of the industry. For example, the seed-stock and commercial producers focus on parental identification, maternal performance, replacement selection, and production efficiency. The feedlot sector relies heavily on improving growth performance, efficiency, animal health, and carcass merit, while the processor is focused on meat quality, carcass value, and food safety (Van Eenennaam and Drake, 2012).

There are many benefits of using genetic testing compared to traditional animal husbandry methodology that focused on phenotypic selection criteria; specifically with traits of low heritability and identification of recessive traits and genetic defects. As the technology continues to evolve and a larger testing population is secured, the accuracy and consistency of genetic selection tools should only increase livestock productivity and efficiency.

IGENITY[®]

Several companies have begun marketing of genetic marker panels to aid producers in the selection of premier traits. One of the more popular products today is sold by Merial Limited (Duluth, GA). IGENITY[®] gene profiles are commercially available to aid in the selection of cattle that better fit their needs of producers and the beef industry. Two types of analysis are available through IGENITY[®]. The first is an index-based scale developed by the company which is presented to producers for each calf tested and reported on a scale of 1 – 10, with 1 being of low improvement potential of a specific trait and 10 being of high improvement potential. The second type of analysis creates estimated molecular breeding values for traits such as hot carcass weight, ribeye area, yield grade, 12th rib fat thickness, percent choice, marbling, tenderness, docility, heifer pregnancy rate, maternal calving ease, and stayability. One of the many goals of IGENITY[®] was to create a “uniform language” that could be understood by all shareholders in the beef production cycle (www.igenity.com). The scores produced by this genetic evaluation are confirmed by the base population that was used to develop the test, consisting of 50,000 head followed from production to the packing plant.

The IGENITY[®] tenderness profile is one that we as researchers have spent much time working with and evaluating. Based on information collected on the company’s website, the range of 1 to 10 for tenderness represents a difference in 2.3 pounds of shear force as measured

through Warner-Bratzler shear force. This differential in selection is the result of identifying genetic markers related to the calpain and calpastatin genes and assigning relative importance of each of these haplotypes to tenderness. Marker panels for all traits continue to evolve, as more research sheds light on the numerous facets involved in each of these expected outcomes. The more genetic knowledge we can obtain from these animals and their genome, the more accurately and effectively we should be able to predict future results. This ability to select livestock based upon predictions of genetic merit gives producers a time advantage over waiting for phenotypic differences to be physically expressed across many offspring. However, we should be cognizant that phenotype is a result of both genotype and environment, and therefore management practices coupled with all the other feedlot and processing plant factors will also contribute to the observed variation in tenderness.

Heritability of Associated Traits

While strides have been made in the use of genetic markers as a selection tool, the most widely utilized selection tools are Expected Progeny Differences (EPD). With regard to carcass merit and quality, EPD's have been utilized for many years to predict genetic differences as a result of sire on measurable traits such as carcass weight, ribeye area, back fat thickness, marbling score, and yield grade or cutability (Crews, 2002). The use of EPD's for carcass traits has long been advantageous, as carcass traits are highly heritable. Conversely, growth and performance traits are moderately heritable, and reproductive traits are low in heritability. From a genetic marker standpoint, it is these traits of low heritability that have the most room for improvement and application of genetic panels.

Within the scientific literature, it is evident that heritability estimates in their own right are variable. This variability can be introduced by any number of factors previously discussed,

as carcass quality can serve as an assessment of that animal's entire life. With that in mind, many of the heritability reference estimates presented will be averages, painting a less cloudy picture of the traits of interest. Starting with palatability traits, a review of 10 manuscripts published by Hocquette et al. (2006) reported the mean heritability estimate for tenderness was 0.24, while juiciness was 0.11, and flavor was 0.09. In the same review using nine studies, the heritability average of intramuscular fat was 0.49. It is important to note that marbling is positively correlated with carcass fatness and therefore selection for increased marbling to improve palatability will also increase the overall fat deposition on the carcass, leading to a decrease in carcass cutability. While this relationship is not as evident as it was many years ago, these two traits are related and striking an optimum balance between the two fat depots presents a challenge to many producers.

While carcass traits are the most prevalent EPD's used today, measurements and predictions of temperament have also been considered. With temperament being reported as a moderately heritable trait, environment is not the only determinant to behavior. Shrode and Hammack (1971) reported a heritability estimate for temperament of 0.40, while Stricklin et al. (1980) reported a value of 0.44 - 0.48. According to these estimates, temperament is a more highly heritable trait than many of the reproductive traits that tend to be between 0.20 and 0.30. In a time when livestock profit margins do not allow for the implementation of genetic marker assisted technology, an understanding of heritability can give producers an alternative option in selecting for not only carcass traits, but also for temperament.

Potential for Genetic Improvement

While genetic testing technology shows numerous benefits to improving selection and ultimately the value of beef cattle marketed today, there is an associated expense. Simple parent

testing costs around \$20/ animal, while genetic profiles can cost as much as \$40/ animal (www.igenity.com). In any livestock industry, added costs need to be followed by a related increase in revenue in order to remain a viable tool. The implementation of technologies such as IGENITY[®] appear to be more easily accepted by larger producers due their ability to effect performance on a scale large enough to show almost immediate returns. Also, these larger producers traditionally have a retained interest in the cattle from birth to market, making it even more beneficial to implement improvements in the herd. Unfortunately, 90% of the cattle ranches in the U.S. have fewer than 100 head, accounting for 46% of the U.S. beef herd (Van Eenennaam and Drake, 2012). Historically, smaller producers tend to be less adaptive to new technologies due to the additional up-front cost. Some of this hesitation may also be the result of limited retained ownership of cattle from conception to market, leaving less incentive to select for end product quality if producers cannot directly see the financial benefits (Van Eenennaam and Drake, 2012). Small producers traditionally market their offspring in sale barns at market price to feedlots rather than feeding them out themselves. This transaction eliminates the ownership the cow/ calf producer has in their calves, making it less likely they will select for traits that will not achieve benefits at this, the front end of the production chain. In the case of the cow/ calf producer, almost all selection pressure can be on reproductive traits and weaning performance, with limited emphasis of feedlot efficiency, carcass merit, or meat quality. Conversely, a percentage of large producers (greater than 500 cows) tend to sell cattle as yearlings or decide to retain ownership through marketing. In the later scenario, the added cost of genetic testing in the cow herd to improve both maternal performance, but also feedlot performance and carcass merit, can be realized in the marketing of those cattle. It is this sector

that is also best positioned to reap the benefits of selection pressure applied to the meat quality parameters if, or rather when, the industry changes to a quality driven carcass payment schedule.

As mentioned, the sector most adamantly looking to genetic assisted selection for improved profitability is the feedlot sector. Genetic selection in this capacity is not positioned to find profitable vs. unprofitable cattle, but rather to fit management strategies to the individual animal. Van Eenennaam and Drake (2012) suggested that feedlot owners were not concerned with how to sort cattle by performance from best to worst, but rather made efforts to use genetic testing to profitably sort cattle into management groups. These management groups could differ by days to market, feed efficiency, or growth performance; allowing for the application of alternative growth promoting implants or feeding strategies to maximize the animal's performance while in the feedlot. Selecting cattle based on different performance or quality parameters could also lead to different marketing strategies that better position cattle for branded and value added programs that could improve the producer's bottom line. However, due to genetic testing still being in the infancy stage, the cost per animal is relatively high and currently does not possess a positive return on investment for any one sector (Van Eenennaam and Drake, 2012). For this technology to ultimately be profitable, the beef industry will need to work together and transfer information across production sectors in order to realize the added benefit of such technologies (Wood, 2011).

Measuring the Influence of Feedlot Temperament

Understanding animal disposition and temperament, and learning how to adapt production strategies to fit the cattle could prove to be advantageous to the producer's bottom line. Cattle that are more restless or temperamental have been shown to perform poorer in the feedlot with lower average daily gains which generally translate into higher overall cost of

production (Tulloh, 1961; Voisinet et al., 1997b). These more excitable cattle are also more difficult to work and can result in added costs because they can require more time, labor, and equipment repair (Hall et al., 2011). From a carcass standpoint, excitable cattle also exhibit a favorable decrease in fat thickness and a lower yield grade which carries along with it the undesirable subsequent decrease in marbling scores and lower USDA quality grades (Reinhardt et al., 2009).

The measurement of beef cattle temperament is still a relatively novel topic within the livestock industry and thus one specific measurement of temperament has yet to be widely accepted by researchers and industry leaders. In order to be a useful tool for evaluating temperament, the method must be reliable, repeatable, and linked to the animal's individual stress response (Curley et al., 2006). This presents a challenge as most measurements used today are subjective, allowing for human error or biased results. A few of the current observational measurements of temperament include exit velocity, pen score, chute score, catch score, hair whorl position, and eye white percentage (Curley et al., 2006; Grandin et al., 1995; Core et al., 2009). With advancing technology in the field of genetic markers, genetic tests are becoming commercially available to categorize temperament of beef cattle at the genetic level with the potential to make subjective analysis obsolete.

Exit velocity

An objective measurement of feedlot temperament is exit velocity. As described by Burrow et al. (1988), exit velocity utilizes infrared motion sensors to record the time it takes an animal to travel a fixed distance. In most literature, this distance is 1.82 m. The "start" sensor is placed at the end of the working chute and the "finish" sensor is placed 1.82 m away. Burrow et al. (1988) reported that faster exit velocity times represent more excitable cattle. From a feedlot

owner's perspective, higher exit velocities have been correlated with reduced average daily gains, thus making exit velocity a potential measurement of cost per gain (Nkrumah et al., 2007; Voisinet et al., 1997b). Hall et al. (2011) reported that cattle exhibiting a slower exit velocity had a higher percentage of kidney, pelvic, and heat fat, as well as a higher marbling score. On the contrary, Nkrumah et al. (2007) reported a negative correlation between exit velocity and final yield grade, where slower exiting cattle had a lower yield grade.

Chute score and Capture Score

Chute score has been utilized as a subjective measurement of cattle behavior while on a weigh scale or similarly confining by a non-restraining device. The chute score system was developed by Grandin (1993) with a score of 1 = calm, no movement; 2 = slightly restless; 3 = squirming, occasionally shaking the chute; 4 = continuous, very vigorous movement and shaking of the chute; 5 = rearing, twisting of the body and struggling violently. Hall et al. (2011) applied the same numeric score to evaluate cattle while the animal was captured in the head gate. Hall et al. (2011) reported a positive correlation between capture score and 36 h postmortem muscle pH (higher capture score correlated to higher intramuscular pH), suggesting a relationship between capture response and muscle glycogen utilization.

Measuring the Influence of Stress at the Slaughter Plant

The measurement and understanding of the effects of pre-slaughter stress is difficult to comprehend due to the inherent variation across animal's arriving at the plant as well as the complex nature of the conversion of muscle to meat. It is difficult yet not impossible to assess an animal's behaviour during the period immediately prior to harvest. Unpublished data collected by Magolski et al. (2012) measured vocalization of cattle prior to stun and collected

blood lactate at exsanguination as an indication of animal stress and metabolic activity (described below).

Vocalization

Grandin (1998) concluded that vocalization scoring is a simple and effective method of detecting welfare problems in the packing plant. The author reported that almost all cattle vocalization events are the direct result of some stressful event, including the use of electric prods, slipping or falling, missed stuns, or excessive squeezing of the chute. Research in hogs showed a similar result, with pig vocalization and squealing level directly related to an increase in the pig's blood lactate concentration (Warris et al., 1994). A benefit of recording vocalization score compared to other forms of animal activity is the objectivity of the vocalization data. A scale developed by Grandin (1998) uses a simple 0 or 1, with 0 equalling no vocalization, and 1 representing vocalization.

Unpublished data by Magolski et al. (2012) showed that cattle with increased vocalization scores more actively resisted the v-belt restrainer and required more time to completely restrain the animal prior to stunning. Additional time in the restrainer may increase the stress load on the animal (as measured by vocalization), potentially having a more negative effect on post-mortem metabolism and meat quality.

Blood lactate

Animals that are more stressed just prior to slaughter undergo a faster rate of anaerobic metabolism, producing an abundant supply of hydrogen ions from hydrolysis of ATP (McVeigh, et al., 1982). This in turns creates an elevated lactate concentration because lactate is responsible for sequestering the available hydrogen ions in an attempt to remove the ions from the system (Scheffler et al., 2011). There is minimal published research evaluating the relationship between

blood lactate and stress in beef cattle; however it has been reported that elevated concentration of circulating blood lactate are associated with increased stress (Mitchell et al., 1988; Voisinet et al., 1997a). Mitchell et al. (1988) reported that lactate spikes are observed in the plasma as a result of handling and transportation stress stemming from stimulation of both the hypothalamic-adrenal cortex phase and the sympathetic-adrenal medulla phase of the sympathetic nervous system response. The authors also identified stunning as a trigger which will result in a massive sympathetic response that results in elevated plasma lactate levels.

While blood lactate is a more available measurement at time of slaughter, tissue lactate levels are also of interest with their relationship to post-mortem metabolism. Qvisth et al. (2008) reported a similar purge of lactate from the muscle during periods of exercise stress, but noted that adipose tissue was also a significant source of lactate release. This increased presence of lactate in adipose tissue results in a spike in lipolysis to aid in energy availability to the muscle. In order for lipolysis to occur an aerobic environment is needed (Romijn et al., 1993), and therefore stress immediately preceding harvest may not directly affect postmortem metabolism and carcass composition. Research attempting to understand a possible postmortem mechanism to relate the effects of pre-slaughter stress with a decrease in quality grade is needed.

Kosher Slaughter

In 2009, the Kosher market contained over 150,000 retail products representing a \$200 billion industry (Hui, 2012). Even with the strong demand, the method of Kosher slaughter by exsanguination of cattle without stunning has long been scrutinized for its deviation in harvest method compared to the commercially acceptable captive-bolt stunning method. Kosher slaughter has long been viewed to have a detrimental effect on animal welfare (Grandin, 2011). The Kosher slaughter method is a Jewish ritual defined as the cutting of the animal's neck to

exanguate them without prior stunning (Grandin, 2010a). A Shochet, or trained Rabbi, uses a long, sharp knife to perform this task while the animal is restrained. Jewish Dietary Laws consider Kosher slaughter to be the most humane method of slaughter to achieve complete draining of the blood from the animal.

While religious slaughter is exempt from USDA regulations published in the Humane Slaughter Act of 1958, much public attention has been focused on the methods of rendering the animal unconscious in this manner. Much of this attention has been the result of undercover videos released from packing plants showing the thrashing and kicking of animals that have been slaughtered via the Kosher method. Grandin (2010b) reported that cattle slaughtered by the Kosher method typically remain conscious for 17 to 85 seconds after a proper throat cut (Grandin, 2010b); compared to immediate unconsciousness with captive bolt stunning. Unpublished data collected by Hayes (2012) showed that some cattle harvested through the Kosher method remain conscious for up to 200 seconds following the throat cut. To reduce this prolonged animal activity, some beef slaughter facilities have elected to follow the throat cut with a captive-bolt stun to reduce animal activity and blood splash (Grandin, 2010a; Hui, 2012).

While some would consider this only an animal welfare issue, unpublished data from Magolski et al. (2012) suggest that it may also be a meat quality issue. These differences in meat quality could be attributed to many different observed measurements that deviate from the traditional expectations associated with captive-bolt stunning. As discussed, time to unconsciousness is much different between the two methods and during this time animal stress and muscular activity may be affecting end product quality. Kosher slaughtered cattle are restrained differently than captive bolt cattle which could result in increased stress just prior to death. With captive-bolt stunning the animal's nose is position downward in a more natural

position, while with the Kosher method, the animal's nose is elevated to expose the neck. This manipulation in head position alone could be an added stressor just prior to exsanguination.

While much meat quality research is focused on minimizing pre-slaughter stress, this may be a stress influencer previously unmeasured.

The duration of time to unconsciousness also presents an opportunity for stress to negatively impact beef quality. Based on personal slaughter floor observations, there are much more visual muscle contractions and unique reactions by animals being harvested via the Kosher method compared to captive-bolt methods. Some of this difference in muscle activity may be a result of the anatomical location of the vertebral arteries. Even with proper severing of these arteries in the neck, the arteries to the brain remain intact, allowing for a prolonged blood supply to the brain (Grandin, 2011). The exacerbated muscle contraction and tension of the animal prior to reaching unconsciousness could have negative effects that last well beyond the slaughter floor. Since most animals are restrained in a v-belt restrainer, much of the observed muscle activity is present in the lateral plane, potentially leading to increased contraction rates of the *longissimus* complex which shorten the muscle fiber length at rigor resulting in more muscle fiber overlap that could lead to a less tender steak (Locker, 1959). While we can speculate on potential changes in meat quality as a result of this slaughter method, there is currently no published literature discussing the differences in slaughter method on beef quality. Some of the presented research in this dissertation, as well as current research being conducted at NDSU, strive to expose any potential adverse meat quality outcomes as a result of the Kosher slaughter method which could lead to preventative measures undertaken by the packing plant to improve the Kosher slaughter process.

One note that seems to be left out of the general conversation when discussing Kosher slaughter is its unintentional impact on the commercial beef cattle market. While many cattle are harvested through the Kosher slaughter method, more than half of the Kosher produced product is marketed through the commercial beef chain. This is due to the fact the hindquarters of these carcasses cannot be marketed as Kosher due to the muscles association with the siatic nerve and are therefore sold in the conventional market (Hui, 2012). It is therefore critical to understand how slaughter method will influence beef quality and palatability as the industry continues to focus on consumer acceptability.

Stress and Beef Quality

Poor temperament observed in the feedlot in the working chutes has been shown to have lasting adverse effects on the subsequent meat product. Some of the most negative effects of temperamental cattle are found in terms of beef tenderness and overall meat quality. Fordyce et al. (1988) reported that as movement, velocity, and overall response to stress increases, so too did the number and size of bruises on the animal. This is a significant financial concern because the 2007 National Beef Quality Audit reported that 35% of cattle marketed in the United States have at least one bruise, while the incidence of multiple bruising was 9.4% (Garcia et al., 2008). According to the National Beef Checkoff, bruising results in a loss of profit of more than \$114 million annually (Slagle, 2007).

Much of the negative influence on meat quality is the result of elevated muscle glycogen depletion during periods of stress as a result of increased heart rate, body temperature, and increased levels of corticosteroids (Warriss, 1990). Warriss (1990) also reported that it takes cattle between 3 and 11 days to fully recover the glycogen levels present prior to the stress. The duration of this recovery period is influenced by sex, availability of feed, and water (McVeigh et

al., 1979). Unfortunately, if these stressful events occur just prior to harvest, a recover period is not possible and can negatively influence meat quality.

Beef Quality

Meat tenderness determined by Warner-Bratzler shear force (WBSF) is influenced by cattle temperament. Voisinet et al. (1997a) reported cattle that were highly agitated and struggled violently in the squeeze chute had significantly higher WBSF values; with 40% of the cattle exhibiting WBSF values above the threshold for acceptability in foodservice distribution (shear force > 3.9 kg). King et al. (2006) also reported that excitable steers had higher ($P < 0.05$) WBSF values than calmer cattle.

Cattle with more excitable temperaments have been reported to have a higher propensity to be borderline dark cutters compared to the calm cattle ($P < 0.01$; Voisinet et al., 1997a). Dark cutting beef, resulting from pre-harvest stress depleting glycogen stores which results in an abnormally high ultimate pH (> 6.0), is an undesirable trait creating a dark, firm, and dry cut lean surface (Lister, 1988). However, King et al. (2006) reported that cattle with calm temperaments had a higher ultimate pH ($P < 0.05$) than those in the intermediate or excitable temperament groups. Other undesirable meat quality traits associated with dark cutting beef include reduced shelf life (Gill and Newton, 1981; Lawrie, 1958) and weak beef flavor (Dransfield, 1981). Factors responsible for the development of this pre-harvest stress, as mentioned prior can include weather, growth promoting implants, genetics, disposition, and handling practices (Hedrick et al., 1959; Smith et al., 1993; Voisinet et al., 1997a). According to the 2005 National Beef Quality Audit representing data collected on 49,330 head, dark cutting beef accounted for 1.9% of the population (Garcia et al., 2008). Deductions for dark cutters included reduction in USDA quality grade by one-third (0.7%), one-half (0.3%), two-thirds

(0.3%), and one full grade (0.5%; Garcia et al., 2008). It is notable that the 2005 Beef Quality Audit showed an overall decrease in reported incidence of dark cutting beef compared to the 2000 Beef Quality Audit figure of 2.3% (McKenna et al., 2002).

While tenderness and pH have been focal point measurements for the ramifications of stress, Ferguson and Warner (2008) concluded that there is a need to broaden the focus to include other traits that may be influenced by stress. The authors suggested that water-holding capacity and subsequent purge should also be evaluated. Data by Warner et al. (2007) showed that cattle under increased stress 15 minutes prior to harvest, induced by electric goads, resulted in an decrease in water-holding capacity and consumer acceptability. These data showed a 21 d purge increased ($P < 0.05$) from 3.5% to 5.4% when the animals were presented with a stress inducing event prior to harvest. Consumer ratings (1-100, 100 being the best) also significantly ($P < 0.05$) decreased from 59.6 to 55.6 when cattle were exposed to added stress.

Another area requiring further research is whether or not there is an effect of pre-harvest stress on adipose tissue, specifically whether or not these stressful events just prior to unconsciousness can induce changes in marbling score. Unfortunately, these data are difficult to standardize in a manner to identify meaningful conclusions. Reinhardt et al. (2009) reported that cattle disposition may be related to the animal's ability to deposit fat, specifically intramuscular fat. However, due to the extensive nature of the traits that influence marbling, they were unable to measure the direct influence of behavior on carcass marbling score.

Responsibilities of the Beef Producer

The beef industry, much like other livestock industries, has multiple stakeholders all with a slightly different production goal, making uniform selection parameters difficult. Garrick and Golden (2009) described these stakeholders as representing the cow-calf, backgrounding,

feedlot, and processing sectors. Each sector focuses on slightly different traits even with the ultimate goal of high quality beef production in mind. For example, the cow-calf producer's goal is to achieve a high percentage calf crop with respectable weaning weights, while the feedlot sectors are most concerned with feed conversion and growth rate. The processors primary goal is to produce a quality product that consumers are willing to purchase; a tender, juicy, and flavorful product of good value. With these traits being at different levels of importance to different industry sectors, it will be important to develop strategies focusing on a combination of traits to move the entire beef sector forward. With that being said, genetic marker assisted selection is a newer area of beef production that will most certainly see more emphasis in the years to come due to its ability to predict differing phenotypes much earlier in life. Decreasing the time needed to make selection decisions will improve the capability of each sector of the marketing chain to achieve their ultimate production goal.

While trait selection will aim to improve beef quality in the long run, improvements in productivity will not solely come from new technologies (Grandin, 2003). Changes in nutrition, growth promoting implant strategies, environmental conditions, and management can all have immediate effects on improving beef quality. At the same time, strategies to minimize livestock stress will greatly improve the quality of the end product. Grandin (2003) reported the best and most efficient means of reducing livestock stress both in the feedlot and the packing plant was to utilize smooth and efficient handling facilities and to ensure that all workers receive proper animal handling training. Not only will management strategies improve product quality, they will also continue to win the respect of the consumer with regard to proper animal welfare and well-being.

Lastly, as beef quality research continues to progress, more attention will need to be focused on traits other than tenderness and pH. As discussed, those traits may include water holding capacity and purge loss, both significant points of economic loss to the packer. New findings would also suggest that slaughter stress needs to be looked at as potentially having negative ramifications on marbling score and subsequent carcass value and palatability. Beyond meat quality however, research should be conducted toward understanding how slaughter practices affect animal stress and eventually meat quality. Preliminary unpublished data would suggest the Kosher slaughter method increases livestock stress, while also having a detrimental effect on meat quality (Magolski et al., 2012). While this religion vs. science discussion is difficult to address, further understanding of these effects is necessary.

Ultimately, livestock production is driven by the producer's bottom line. While farm income is as important as ever, it is the consumer who will decide whether or not we stay in business. Supplying to the consumer's needs in regards to cattle welfare and product palatability at a fair price while continue to ensure profitable marketing of beef well into the future. While producers may implement technologies and management practices to improve their position in the industry today, the application of these new technologies and management practices are an investment in the future perceptions and purchasing decisions of the United States beef consumer.

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**CHAPTER II. RELATIONSHIP BETWEEN GENETIC EVALUATION
TECHNOLOGIES AND PHENOTYPIC OBSERVATIONS ON BEEF QUALITY
AND TENDERNESS**

Abstract

Warner-Bratzler shear force values from 570 mixed breed heifers and steers were used to determine estimates of genetic selection. Cattle used for this analysis were marketed from 2008 to 2011, and included five different feedlot based research projects at the North Dakota State University- Carrington Research Extension Center (Carrington, ND). Represented breeds include Angus, Simmental, Charolais, Shorthorn, Hereford, Chianina, Gelbvich, Maine-Anjou, and Piedmontese. Samples were collected for IGENITY[®] (Merial Limited, Duluth, GA) analysis providing information that included selection indices and breeding values for the following genetic traits: hot carcass weight (IHCWT, BVHCWT), ribeye area (IREA, BVREA), yield grade (IYG, BVYG), fat thickness (IFT, BVYG), percent choice (IPCH, BVPCH), marbling (IMARB, BVMARB), tenderness (ITEND, BVTEND), docility, heifer pregnancy rate, maternal calving ease, and stayability. These genetic-based parameters were compared with actual measurements including (HCWT), ribeye area (REA), yield grade (YG), fat thickness (FT), dressing percent (DP), marbling (MARB), average daily gain (ADG), colorimeter color scores (L*, a*, and b* values), Warner-Bratzler shear force (WBSF) values, and measurements of temperament including exit velocity (EV), chute score (CS), and capture score (CAPS). Four direct traits of influence on tenderness included hue angle (HA), YG, MARB, and BVTEND. Marbling accounted for over 10% of the variation in WBSF, and HCWT was the second most influential carcass trait accounting for 4% ($P < 0.01$). Overall, regression coefficients of IGENITY[®] molecular breeding value on phenotype for WBSF, MARB, REA, YG, and FT were

relatively low ($R^2 = 0.14, 0.02, 0.03, 0.03, \text{ and } 0.02$, respectively). These data suggest that selecting cattle for a higher degree of marbling and feeding them appropriately is the most important factor influencing beef tenderness and acceptability.

Introduction

One of the most important factors influencing the acceptability of beef, and the most studied palatability trait of cooked meat, is tenderness (Beermann, 2009). There are several reasons for the continued focus on tenderness due to the many biological factors that affect conversion of muscle to meat and the development of tender meat. Some of these influential factors include genetics, time on feed, nutrition, growth promotants, age, stress, chilling rate, and aging of the product (Tatum et al., 2007). Marbling has also been shown to have a small but positive influence on tenderness as well as juiciness and flavor (Wheeler et al., 1994). It has been a challenge for producers to manage production factors that could lead to improving tenderness because of the large variety of phenotypic traits that influence tenderness. Tenderness remains an issue at the production level because there is a financial incentive involved with improving tenderness due to the potential of higher price incentives that consumers are willing to pay for guaranteed tender (Boleman et al., 1997). Genetic testing is one technology that has surfaced in the commercial market within the last 10 years to help producers with these selection decisions effecting economically significant traits.

IGENITY[®] (Merial Limited, Duluth, GA) gene profiles are commercially available to aid producers in the selection of cattle that better fit their needs and those of the industry. Two types of analysis are available from the IGENITY[®] company, including the index based results presented on a scale of 1 – 10, with 1 being of low improvement of a specific trait, and 10 being of high improvement potential. The other type of analysis is an estimated molecular breeding

value for traits such as hot carcass weight, ribeye area, yield grade, 12th rib fat thickness, percent choice, marbling, tenderness, docility, heifer pregnancy rate, maternal calving ease, and stayability. The ability to select livestock based upon predictions of genetic merit gives producers a time advantage over waiting for phenotypic differences to be collected from offspring. However, phenotype is a result of both genotype and environment and therefore management practices do contribute to variation in tenderness.

The objective of this project was to evaluate the correlations between actual carcass measurements and genetic-based evaluations and to evaluate the relationship between the economically viable traits of interest. The applied nature of this research is to assist producers with understanding methods and strategies to assist in selecting cattle that best fit their needs and the needs of the consumer.

Materials and Methods

Description of the Data

This study is the result of five separate but related studies evaluating feedlot performance, temperament, carcass traits, and meat quality parameters of 570 crossbred steers and heifers. All cattle were finished at the North Dakota State University- Carrington Research and Extension Center (CREC; Carrington, ND). Breed type crosses represented included Angus, Red Angus, Simmental, Charolais, Piedmontese, Hereford, Gelbvieh, South Devon, Chianina, Maine Anjou, and Shorthorn.

Diets and Treatments

Study one evaluated the effect of feedlot temperament on meat quality and postmortem protein degradation on 182 mixed composition steers. Breed type crosses represented included Angus, Red Angus, Charolais, Hereford, Simmental, South Devon, Gelbvieh, Maine Anjou,

Chianina, and Shorthorn. These cattle were in the CREC feedlot from October of 2007 to May 2008. Study two was a finishing period comparison study of natural vs. conventional beef feedlot feeding strategies and their effect on behavior and meat quality using 78 yearling Angus steers. These data were collected from March of 2008 to May of 2008. Study three utilized 131 Angus and Angus cross yearling heifers to evaluate the effect of glycerol inclusion (0, 6, 12, or 18%) in the receiving phase on growth rate and meat quality parameters and was conducted from June of 2008 through September of 2008. Study four took place from June of 2009 to September of 2009 using 58 Angus and Angus cross yearling steers to understand the effect of replacing corn with pea hulls or pea chips in finishing diets on meat quality. Lastly, project 5 conducted from June of 2010 to September of 2010 evaluated the effect of different field pea components in the finishing diet of 121 Angus x Piedmontese heifers on meat quality parameters. Dietary treatments included the replacement of corn with field peas, pea hulls, or pea chips. Project hypotheses were formulated using regional co-products as a local alternative to corn with the potential to improve feedlot performance and meat quality parameters. In all projects there was no significant influence of dietary treatment on feedlot performance, carcass composition, marbling, color score, or WBSF.

Feedlot Data Collection

Weights were obtained and measurements of temperament including exit velocity (EV), chute score (CS), and catch score (CAPS) were recorded throughout the feedlot phase. Within each project, cattle were moved from their home pens to the working chute, pen by pen, by the same livestock technicians each time. Cattle were moved through the working chute by the same employees each time using rattle paddles as necessary. Exit velocity was measured as described by Burrow et al. (1988) using infrared motion sensors (Farm Tek, Inc., Wylie, TX). The “start”

sensor was placed approximately 0.5 m from the end of the working chute (head gate) and the “finish” sensor was placed 1.82 m away. Exit velocity was recorded as the time it took each animal to travel the 1.82-m distance and converted to meters/second. Chute score was visually observed and assigned while cattle were on the weigh scale with both entry and exit gates closed. The CS system was developed by Grandin (1993) with a score of 1 = calm, no movement; 2 = slightly restless; 3 = squirming, occasionally shaking the chute; 4 = continuous, very vigorous movement and shaking of the chute; 5 = rearing, twisting of the body and struggling violently. Cattle were not restrained while on the weigh scale (SenseTek, Saskatoon, SK). The CAPS was recorded utilizing the same numeric scale (1 to 5) as CS, however, this evaluation was recorded based on activity while the animal was captured in the head gate. The subjective observations (CS and CAPS) were evaluated by the same technician throughout the duration of the experiments from the same vantage point.

IGENITY[®] collection

IGENITY[®] (Merial Limited, Duluth, GA) tissue collections were conducted on each animal using an ear tag punch purchased through IGENITY[®]. Samples were then sent to Lincoln, NE for analysis. Two types of analysis were utilized for each animal. One form of analysis was index based with results presented on a scale of 1 – 10, with 1 being of low improvement potential of a specific trait and 10 for high improvement potential. The other type of analysis provided an estimated molecular breeding value for each animal. Traits analyzed by IGENITY[®] included hot carcass weight (HCWT), ribeye area (REA), yield grade (YG), 12th rib fat thickness (FT), percent choice (%CH), marbling (MARB), tenderness (TEND), docility, heifer pregnancy rate, maternal calving ease, and stayability.

Carcass data

At approximately 14 to 16 months of age cattle were delivered to a commercial packing facility. Feed was withheld from all cattle roughly 12 h prior to loading. Cattle in project 1 were loaded into five drop-center double deck trailers and transported 746 km (8 h travel time) for humane slaughter at Tyson Foods (Dakota City, NE). Cattle in projects 2, 3, 4, and 5 were transported on drop-center double deck trailers and transported 26 km (20 m travel time) for humane slaughter at North Dakota Natural Beef (New Rockford, ND). Lairage time averaged 2 h across projects. Carcass measurements including HCWT, REA, YG, FT, and MARB were obtained at approximately 24 h postmortem. At 24 h postmortem, a 7-cm thick *longissimus dorsi* sample was obtained caudal the 12th rib, placed in a labeled Ziploc bag, placed in a cooler, and transported to the North Dakota State University Meat Lab (as previously described by Hall et al., 2011). Meat samples were unpacked and deboned at the NDSU Meat Lab upon arrival. A 2.54-cm thick boneless strip steak was cut from the collected sample for use in Warner-Bratzler shear force (WBSF) measurement. Color was measured using a Minolta Chroma-meter (Konica Minolta, Grand Rapids, MI) to record L* (lightness/darkness), a* (red/green), and b* (yellow/blue) values from each steak after approximately 15 min bloom time (Wulf and Wise, 1999). After aging 14 days, each steak was then measured for WBSF following AMSA (1995) procedures.

Statistical Analysis

Statistical analysis was conducted using least squares procedures (GLM, REG), taking into account variation due to year, project, treatment, sex, and slaughter method. Pooled within-class correlations among all traits of importance were obtained. A model was developed to illustrate relationships for all traits influencing tenderness and standard partial regression

coefficients were obtained so that the relative importance of each trait on tenderness could be determined. Correlations and standard regression coefficients were also calculated for each breeding value with its analogous phenotypic value. Estimates of heritability were obtained as the regression of IGENITY[®] molecular breeding values on phenotypic values.

Results and Discussion

Traits analyzed by IGENITY[®] included HCWT, REA, YG, FT, %CH, MARB, TEND, docility, heifer pregnancy rate, maternal calving ease, and stayability. These genetic predictions were compared with the animal's actual measurements including HCWT, REA, YG, FT, dressing percent (DP), MARB, ADG, Minolta color scores including L*, a*, and b*, WBSF, and measurements of temperament including EV, CS, and CAPS.

A summary of IGENITY[®] index values (average and range) for TEND (ITEND), MARB (IMARB), REA (IREA), YG (IYG), HCWT (IHCWT), and FT (IFT) are presented in Table 2.1 among each project. All projects contained cattle with a relatively high propensity for a low WBSF value and a high marbling score. The Angus breed influence in projects 2, 3, 4, and 5 can be observed through the high ITEND, IMARB, IYG, and IFT values along with relatively low IREA and IHCWT indices. IGENITY[®] TEND values for project 1, 3, and 5 are the only traits throughout the data set where all index values (1-10) are represented. Project 1 range of indices shows the diversity of breeds represented, while project two indices show those 78 head had the highest potential for TEND, MARB, FT and YG, while also representing the lowest potential HCWT. We could assume a higher proportion of British influence in this group relative to the other four projects was a contributing factor for these data.

Table 2.1. Project average (range) of IGENITY® index values for tenderness (ITEND), marbling (IMARB), ribeye area (IREA), yield grade (IYG), hot carcass weight (IHCWT), and back fat thickness (IFT).

Project	ITEND	IMARB	IREA	IYG	IHCWT	IFT
1 ^a	4.95 (1-10)	6.87 (3-10)	5.08 (2-9)	6.02 (3-8)	2.93 (1-8)	6.27 (3-9)
2 ^b	7.49 (3-10)	7.61 (5-10)	3.61 (2-5)	7.62 (6-9)	2.16 (1-4)	7.92 (6-10)
3 ^c	5.65 (1-10)	6.24 (3-9)	4.79 (3-8)	5.96 (3-9)	6.31 (4-9)	5.42 (4-8)
4 ^d	5.50 (3-10)	5.88 (3-8)	4.83 (3-8)	6.04 (3-8)	NA	5.99 (3-8)
5 ^e	5.58 (1-10)	6.31 (4-8)	4.76 (3-7)	6.11 (4-8)	NA	5.42 (4-8)

^a 182 mixed composition steers; marketed May of 2008.

^b 78 mixed composition steers; marketed May of 2008.

^c 131 mixed composition heifers; marketed September 2008.

^d 58 mixed composition steers; marketed September 2009.

^e 121 Angus x Piedmontese heifers; marketed September 2010.

Table 2.2 includes the measured carcass values for each of these traits. An average WBSF value for all 5 projects was below 4.0 kg. According to Boleman et al. (1997), these WBSF averages are all categorized as “tender,” representative of the breeds utilized. Average project MARB values (small to modest; low choice to average choice) were also higher than the reported industry average today (slight, high select; Garcia et al., 2008). Actual values show that cattle from project two did have the lowest WBSF value and the smallest REA. Also important to note is that project 2 average carcass weight was the lowest of all project groups, however hot carcass weight was significantly different between the two treatments groups (natural vs. conventional), and was credited to a decrease in growth performance of the cattle on the natural treatment.

Figure 2.1 includes the path coefficient model relative to those traits that have a statistically significant and direct influence on tenderness. Based upon our measured parameters and understanding of the physiological and metabolic effects on tenderness, the four direct traits

of influence on tenderness include hue angle (HA), YG, MARB, and the breeding value for tenderness (BVTEND). Yield grade was included in the model based on the presence of HCWT, REA, and FT in the determination of YG, however, in our data set, YG alone did not indicate an effect on tenderness ($r = -0.002$). With HCWT serving as an indicator of finish weight and maturity, we expected to see a stronger relationship between HCWT and tenderness, since WBSF values have been reported to increase with increasing maturity (Van Koevinger et al., 1995). Similar findings were observed with REA, as we would expect REA to serve as a reflection of carcass merit potential and breed influence, both having a strong influence on tenderness as suggested by Campion et al. (1975) who observed that larger REA was associated with an increase in WBSF values.

Table 2.2. Project average (range) of measured carcass traits for tenderness (WBSF), marbling score (MARB), ribeye area (REA), yield grade (YG), hot carcass weight (HCW), and back fat thickness (FT).

Project	WBSF	MARB ^f	REA	YG	HCWT	FT
1 ^a	3.78 (2.06-9.66)	303 (147-524)	13.7 (9.5-18.1)	2.7 (1.0-5.5)	824 (639-1079)	0.42 (0.03-0.98)
2 ^b	3.09 (1.95-5.21)	417 (230-644)	11.6 (8.8-14.3)	3.2 (1.4-5.5)	675 (508-800)	0.55 (0.15-1.34)
3 ^c	3.39 (1.90-5.70)	458 (311-744)	13.4 (10.0-17.0)	3.4 (1.5-4.5)	806 (621-951)	0.61 (0.24-1.23)
4 ^d	3.27 (1.80-5.83)	405 (280-670)	14.3 (9.8-20.5)	2.8 (0.5-5.1)	785 (631-924)	0.56 (0.24-0.96)
5 ^e	3.99 (2.92-6.65)	330 (200-570)	12.6 (9.7-15.2)	2.7 (1.0-4.0)	719 (625-850)	0.43 (0.16-0.72)

^a 182 mixed composition steers; marketed May of 2008.

^b 78 mixed composition steers; marketed May of 2008.

^c 131 mixed composition heifers; marketed September 2008.

^d 58 mixed composition steers; marketed September 2009.

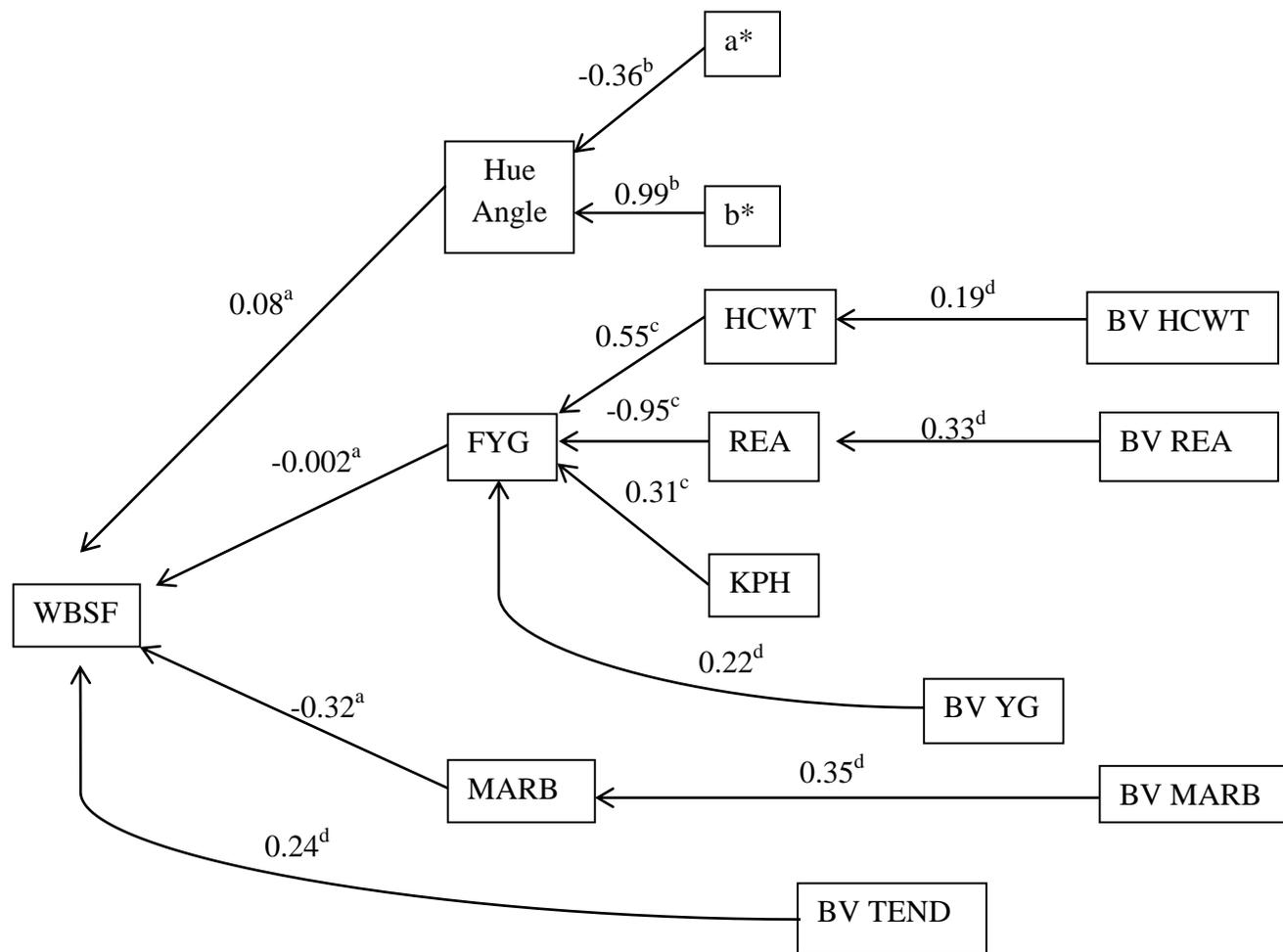
^e 121 Angus x Piedmontese heifers; marketed September 2010.

^f Marbling Score numeric designation: 100 = traces; 200 = slight; 300 = small; 400 = modest; 500 = moderate, 600 = slightly abundant; 700 = moderately abundant.

Hue angle ($HA = \tan^{-1} [b^*/a^*]$), or true red, was calculated from the standard equations (Minolta, 1994) and included in the model rather than a^* , b^* , or chroma (color saturation) for two reasons. Since hue angle is a more objective and relatable trait of fresh meat, we believe the implications are more relevant to the reader. Hue angle represents the change from the true red axis, with increasing values representing a shift to a more yellow pigmentation. Rentfrow et al. (2004) described hue angle as the “true” nature of color beginning at the positive a^* axis and revolving 360° around the three dimensional color space, whereby 0° would be true red (positive a^*), 90° would be true yellow (positive b^*), 180° would be true green (negative a^*), and 360° would be true blue (negative b^*). Secondly, both a^* and b^* showed small but significant influences on tenderness, and the utilization of hue angle takes into account both measured values.

Figure 2.1 also depicts the relationship between BVTEND and WBSF. A partial regression coefficient of 0.24 would indicate that BVTEND accounts for approximately 6% of the observed variation in WBSF. This small, but significant ($P < 0.001$) value of accountability should be of no surprise due to the extensive list of environmental factors known to affect tenderness. Tatum et al. (2007) listed numerous factors that affect tenderness including genetics, time on feed, nutrition, use of growth promotants, stress, age, chilling rate, state of muscle contraction, proteolytic degradation by the calpain system, and amount of connective tissue. Even though producers can select cattle that are expected to excel in a given trait based on genetic heritage, our data would suggest the impact of environment has a much stronger effect on the end result. Current findings indicate MARB accounts for over 10% of the variation in WBSF, with HCWT being the second most influential carcass trait accounting for approximately 4% of the variation in WBSF. Most research suggests a range of 5-10% with regard to the

Figure 2.1. Pathway Coefficient diagram presenting the direct and indirect effectors on Warner-Bratzler Shear Force values (WBSF) and relative regression coefficients for each relationship.



^a Standard partial regression coefficients with Hue Angle, FYG, and MARB as x variables and WBSF the y variable in the model.

^b Standard partial regression coefficients with a* and b* as x variable and Hue Angle the y variable in the model.

^c Standard partial regression coefficients with HCWT, REA, and KPH as x variables and FYG the y variable in the model.

^d Standard regression coefficients between measured traits and the associated breeding values.

variation in tenderness accounted for by marbling (Wheeler et al., 1994; Parrish et al., 1973). Current results suggest that marbling is the single most influential factor for tenderness, representing the upper range presented at 10.5%. Alternatively, Devitt et al. (2002) reported that marbling plays an important role in the juiciness and flavor of beef, however a limited role in tenderness.

Devitt et al. (2002) also pointed out that much of the observed variation in tenderness is independent of marbling and these non-marbling factors led the authors to question the value of USDA quality grade as a predictor of palatability (USDA, 1989). Wheeler et al. (1994) cited that much of the problem with quality grade is that it does not sufficiently segregate carcasses by palatability, as there is much variation in tenderness within quality grade assignments. This variation can be partially accounted for by genetics, age at slaughter, post-mortem aging, and cooking method (Devitt et al., 2002). We agree with the recommendation that a more direct measure of meat tenderness is needed to ensure desirable palatability ratings by the consumer, however, based on our data and those published by many others, marbling score appears to be the best measurement currently available.

Since the mapping of the bovine genome, numerous genetic tools have become commercially available to assist beef producers in the selection of economically important traits (Mujibi et al., 2001). One of these tools is marker assisted selection (MAS) available from such companies as IGENITY[®]. Marker assisted selection is based on the molecular breeding values of the individual animals, considered to be the weighted sum of the number of copies of the frequent alleles of several polymorphisms estimated in a referenced data set (Kachman, 2008). Most of the variation associated with molecular breeding values relative to any one breeding value that was accounted for in an associated observed trait was 13% (MARB), with the lowest

being around 3.5% (HCWT). The breeding value for WBSF accounted for about 6% of the variation in actual WBSF values. Marbling, which exhibited the most influence on WBSF, also showed the strongest relationship between the breeding value and actual value. The strength of this relationship was somewhat surprising knowing the influence of environmental factors influencing marbling, including breed, nutrition, and stress. Dekkers (2007) reported that molecular breeding values only account for a small percentage of the total genetic variance, as polygenic values are also needed to better utilize genetic based selection. Crews et al. (2008) suggested that for a marker panel to be useful it would have to account for 10-15 % of the genetic variation in a given trait. The use of genetic selection to improve tenderness is a valid and important tool to utilize and is one where progress can be made. At the same time, producers should understand these selection parameters are based on genetic characteristics and that environmental conditions will also play an important role in the development of the end product. Interestingly, MacNeil et al. (2010) reported that such genomic selection tools to predict production performance in dairy cattle have been successful, but unfortunately, this success has not been achieved yet in the beef cattle population.

The associated partial correlation coefficients and level of significance are presented for each level of variables influencing WBSF. Table 2.3 includes the partial correlation coefficients between the three primary factors effecting WBSF including HA, YG, and MARB. Based on the known relationship between fat deposition and YG, we may expect a stronger relationship between MARB and YG, however, these values give credence to the fact that modern beef genetics are designed to achieve a higher degree of marbling at a lower YG. Information from the current research suggests that improvements in genetic selection have minimized the parallel relationship between increasing marbling and increasing yield grade, ultimately reducing the

notion that higher marbling cattle have reduced cutability (Koch et al., 1979). Table 2.3 also includes the relationship between the three primary factors influencing YG, including HCWT, REA, and KPH. We observed a positive correlation ($r = 0.44$) between HCWT and REA which would be expected, even with such breed diversity present.

Table 2.3. Partial correlation coefficients and level of significance for A) the primary factors effecting Warner-Bratzler shear force values including hue angle, final yield grade (FYG), and marbling; and for B) the primary factors effecting final yield grade (FYG) values including hot carcass weight (HCWT), ribeye area (REA), and % kidney, pelvic, and heart fat (KPH).

A)	FYG	Marbling	B)	REA	KPH
Hue Angle	0.19 (< 0.0001)	0.19 (< 0.0001)	HCWT	0.44 (< 0.0001)	0.23 (< 0.0001)
FYG		0.32 (< 0.0001)	REA		0.08 (-0.08)

Presented in Table 2.4 are the partial correlation coefficients and levels of significance for breeding values of HCWT, REA, YG, MARB, and TEND. Once again, the relationship between MARB and TEND was observed in the measured values. An increase in breeding value for MARB resulted in an increase breeding value for TEND ($r = -0.15$), implying that as MARB increases, the expected WBSF value will decrease, resulting in a more tender product.

Table 2.4. Partial correlation coefficients and level of significance for the relationship among breeding values associate with beef quality including hot carcass weight (BVHCWT), ribeye area (BVREA), yield grade (BVYG), marbling score (BVMARB), and tenderness (BVTEND).

	BVREA	BVYG	BVMARB	BVTEND
BVHCWT	0.44 (< 0.0001)	-0.2 (< 0.0001)	0.01 (0.84)	-0.02 (0.72)
BVREA		-0.54 (< 0.0001)	-0.21 (< 0.0001)	0.25 (< 0.0001)
BVYG			0.36 (< 0.0001)	-0.26 (< 0.0001)
BVMARB				-0.15 (0.001)

Consistently, carcass traits are of high heritability, while traits of reproduction are less heritable, and some traits such as days to puberty or first breeding are not heritable. Using the

regression of molecular breeding values on phenotype (RMBVP) as an indicator of heritability, evaluation of information presented in Table 2.5 suggests a very low RMBVP coefficient for measured traits in the current data including TEND, MARB, REA, ADG, YG, HCWT, and FT. The highest RMBVP value in the current analysis was associated with WBSF ($r = 0.14$). While RMBVP is not a direct measurement of heritability due to the lack of sire estimates, the value does give us insight regarding the relationship between genetic and phenotypic parameters.

Table 2.5. Regression coefficient of IGENTIY[®] molecular breeding value on phenotype for tenderness (WBSF), marbling score (MARBLING), ribeye area (REA), average daily gain (ADG), yield grade (YG), hot carcass weight (HCWT), and fat thickness (FT) using group as the class variable.

Trait	Regression Coefficient
WBSF	0.14
MARBLING	0.02
REA	0.03
ADG	0.005
YG	0.03
HCWT	0.006
FT	0.02

The mean heritability estimates for WBSF in most literature is reported to be moderately high at 0.29 (Dikeman et al., 2000); however Van Vleck et al. (1992) reported this value to be 0.06, while Barkhouse et al. (1996) reported WBSF heritability estimates as low as 0.02. With some published heritability values for WBSF not significantly different from zero. Based on the results of the former studies, we could assume these populations have a limited genetic variance, and consequently, selection for tenderness would result in little improvement (Barkhouse et al., 1996). It is also important to note that there are several different methods utilized to calculate heritability, therefore lending itself to further variance. This variation in published heritability estimates leaves us to question how changes in modern breeding programs have influenced the potential selection for palatability and tenderness.

With regard to heritability of marbling, Van Vleck et al. (1992) reported a value of 0.43. Other heritability estimates of marbling reported ranges from 0.12 (Shanks et al., 2001) to 0.76 (Thallman, 2004). With these values much higher and more variant than those of WBSF, there is more justification for selection potential of marbling as the industry moves forward. Based on the observed relationship between marbling and tenderness in the present study and others, more sire selection pressure to improve MARB could ultimately improve WBSF values. Conversely, others suggest marbling is a poor predictor of palatability, especially for muscles not associated with the *longissimus thoracis* (Smith et al., 1984; Wheeler et al., 1994).

Ribeye area and YG had the next highest RMBVP value at 0.03, which most comparable literature would categorize as not very heritable. These results may suggest two possible responses. First, even though scientists consider carcass traits to be moderately heritable ($h^2 \geq 0.35$) in most livestock species (Utrera and Van Vleck, 2004), we must always consider the influence of environmental variation such as days of feed, plane of nutrition, and stress. Secondly, these data emphasize the importance of using genetic markers to improve beef cattle selection in order to isolate cattle that have the potential to improve the herd based on ideal environmental conditions. Once these superior animals have been identified, management of environmental effectors such as nutrition, handling, and stress will assist that animal in reaching its maximum potential.

Measurements of temperament including EV, CS, and CAPS were not related to WBSF and therefore not included in the pathway model. The lack of significance was due to the variation in data collection across the projects. Hall et al. (2011) reported that initial EV and CAPS are the best predictors of WBSF. In the current project, these initial measurements were only collected in project 1 and 2. Project 3, 4, and 5 temperament scores were obtained

immediately prior to marketing (final). Hall et al. (2011) reported that most cattle acclimate to the working facilities over time, and therefore final temperament scores do not accurately assess cattle behavior in a novel environment. Initial temperament scores of all cattle would have been beneficial to incorporate into the model as another primary effector.

Implications

Understanding the relative importance of specific traits on tenderness is extremely important for improving the quality of U.S. beef because tenderness is the single most important factor influencing consumer acceptability and U. S. consumers are willing to pay more for guaranteed tender beef. These present analyses reinforce the benefits of selecting cattle with the genetic predisposition toward a higher degree of finish to improve the value of the carcass, and to insure a better eating experience for the consumer. Sorting carcasses using USDA quality grade as a standard measure of palatability is still one of the best assessments available to ensure an acceptable product. Our data suggests that selecting cattle that will have a higher degree of marbling and feeding them appropriately to reach that potential is the most important factor influencing beef tenderness. Ultimately, livestock production is driven by the producer's bottom line, but at the same time if we ensure consumer acceptability and palatability, producers are making an investment in the future perceptions and purchasing decisions of the beef consumer.

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**CHAPTER III. DIFFERENCES IN MINOLTA COLOR SCORE AND
BEEF TENDERNESS ASSOCIATED WITH FEEDLOT STRESS AND
SLAUGHTER METHOD**

Abstract

The objective was to investigate the effect of beef cattle temperament and slaughter method on Minolta color scores and tenderness. Measurements of temperament including exit velocity (EV), chute score (CS), and capture score (CAPS) were obtained prior to slaughter on Angus x Piedmontese crossbred heifers (n = 126). Heifers were slaughtered on two consecutive Mondays (64 and 62 head, respectively) using either Kosher or captive bolt slaughter methods. Climatic conditions and transportation method and duration were similar between slaughter dates. Vocalization (VOCAL) scores were assigned while in the v-belt restrainer and blood lactate concentration (LAC) was measured approximately 40 s after exsanguination. At approximately 24 h post-mortem, carcass measurements and marbling scores were obtained. *Longissimus thoracis* (LT) samples were collected and aged 14 d prior to Minolta color score and Warner-Bratzler shear force (WBSF) measurements. Chute score, CAPS, and VOCAL significantly correlated ($P < 0.03$) with LAC. The LT from kosher slaughtered heifers had significantly higher ($P < 0.01$) L*, a*, b* and WBSF values than that of captive bolt stunned heifers. The LT from captive bolt stunned heifers had significantly higher ($P = 0.04$) marbling, and a tendency ($P = 0.08$) for increased cook loss compared with that from Kosher slaughtered heifers. These data indicate that chute behavior is significantly correlated to measurements of LAC and suggests that the Kosher slaughter method may affect Minolta color score values, decrease tenderness and marbling, and reduce cook loss in LT when compared with the captive bolt stunning method.

Introduction

Beef tenderness is the most studied and most variable palatability attribute of cooked beef (Beermann, 2009; Weaver et al., 2008). The continued focus on tenderness is due to the vast number of factors influencing tenderness and the consumer's request for a tender product. Research suggests increased stress in the feedlot, during transportation, and at the slaughterhouse can lead to increased bruising, decreased tenderness, and increased risk of dark cutters (Voisinet et al., 1997). These negative quality traits can influence profitability and the consumer's willingness to purchase beef. Cattle that possess a mild disposition have improved efficiency in the feedlot and produce a more consumer acceptable product (Nkrumah et al., 2007).

In 2009 the Kosher market contained over 150,000 retail products representing a \$200 billion industry (Hui, 2012). Even with strong demand, the method of Kosher slaughter by exsanguination of cattle without stunning has long been scrutinized for its deviation in harvest method compared to captive bolt stunning. Kosher slaughter has also been reported as having a detrimental effect on animal welfare (Grandin, 2011). The current study was conducted to evaluate the influence of feedlot and pre-slaughter temperament, as well as slaughter method, on meat color and tenderness. A second objective was to evaluate the differences in meat quality resulting from Kosher and captive bolt stunning methods.

Materials and Methods

Animals

All methods and procedures were reviewed and approved by the North Dakota State University Animal Care and Use Committee. Feedlot temperament data was collected on September 9, 2010 on 126 Angus x Piedmontese heifers located at the Carrington Research Extension Center (CREC; Carrington, ND). Exit velocity was measured as described by Burrow

et al. (1988) using infrared motion sensors (Farm Tek, Inc., Wylie, TX). The “start” sensor was placed approximately 0.5 m from the end of the working chute (head gate) and the “finish” sensor was placed 1.82 m away. Exit velocity was recorded as the time it took each animal to travel the 1.82-m distance and converted to meters/second. Chute score was visually observed and assigned while cattle were on the weigh scale with both entry and exit gates closed. The CS system was developed by Grandin (1993) with a score of 1 = calm, no movement; 2 = slightly restless; 3 = squirming, occasionally shaking the chute; 4 = continuous, very vigorous movement and shaking of the chute; 5 = rearing, twisting of the body and struggling violently. Cattle were not restrained while on the weigh scale (SenseTek, Saskatoon, SK). The CAPS was recorded utilizing the same numeric scale (1 to 5) as CS, however, this evaluation was recorded based on activity while the animal was captured in the head gate. The subjective observations (CS and CAPS) were evaluated by the same technician throughout the duration of the experiments from the same vantage point.

Carcass Data

At approximately 14 to 16 months of age (580 ± 43 kg BW), heifers were transported 26 km to North Dakota Natural Beef (New Rockford, ND) where they were humanely slaughtered on 2 consecutive Mondays (September 13 and 20; 64 and 62 heifers, respectively) with 53 of the 64 on d 1 harvested Kosher, and the remaining 73 stunned using a captive bolt. Feed withdrawal (12 h), Climatic conditions, transportation method, transportation time, and lairage time were similar between harvest dates. Vocalization scores (0 = no vocalization, 1 = little vocalization, 2 = extensive vocalization) were assigned from entry into the v-belt restrainer until time of stunning or exsanguination. Approximately 40 s after exsanguination, a 2 ml blood sample was collected for LAC (Lactate Pro Meter, Arkray USA, Inc., Edina, MN).

Carcass measurements were obtained approximately 24 h postmortem at North Dakota Natural Beef (Fargo, ND) and included hot carcass weight (HCWT), 12th rib fat thickness (FT), ribeye area (REA), kidney, pelvic, and heart fat percentage (KPH), marbling (MARB), and yield grade (YG). A 2.54 cm-thick strip steak was obtained at the 13th rib, placed in a labeled Ziploc bag, packed in a cooler, and transported to the NDSU Meats laboratory. Upon arrival, steaks were removed from the Ziploc bag and allowed to bloom for approximately 15 min (Wulf and Wise, 1999). Color was measured using a Minolta Chroma-meter (Konica Minolta, Grand Rapids, MI, USA) to record L*, a*, and b* for each strip steak. After aging 14 d in individual vacuum sealed Cryovac[®] (Duncan, SC) bags at 3° C, each steak was measured for WBSF following AMSA (1995) procedures.

Statistical Analyses

Data was analyzed using Proc CORR and Proc GLM procedures of SAS (SAS Institute Inc., Cary, NC) with slaughter method x slaughter date and pen within slaughter date in the model. These procedures accounted for variation as a result of two different slaughter days, two slaughter methods, and the cattle being group housed in 16 total pens.

Results and Discussion

Feedlot and Slaughter Stress

The relationship between feedlot temperament and pre-slaughter measurements is reported in Table 3.1. Temperament scores including CS, CAPS, and VOCAL correlated ($P < 0.03$) with LAC ($r = 0.267$, $r = 0.249$, and $r = 0.369$, respectively). The strongest correlation was between VOCAL and LAC.

Animals that are more stressed just prior to slaughter undergo a faster rate of anaerobic metabolism (Warriss, 1990), increasing hydrogen ion availability from ATP hydrolysis. This in

turn elevates blood lactate concentration, as lactate is responsible for hydrogen ion sequestering in an attempt to remove the ions from the system (Scheffler et al., 2011). This direct relationship between VOCAL and LAC at time of exsanguination can also be observed in Figure 3.1. Cattle with a VOCAL score of 0 had a significantly ($P = 0.001$) lower LAC compared to cattle that exhibited excessive vocalization (VOCAL = 2; 9.53 vs. 12.99 mmol/ L respectively). As a result of these data, LAC at time of slaughter can potentially serve as an objective measurement of cattle stress during the ante-mortem process.

Table 3.1. Partial correlation coefficients (P -value) for Warner-Bratzler shear force (WBSF), chute score (CS), capture score (CAPS), exit velocity (EV), vocalization prior to slaughter, blood lactate concentration (mmol/L) at time of slaughter, and L*, a*, and b* Minolta color scores.

Item ^a	Chute Score	Capture Score	Exit Velocity	Vocalization	L* ^b	a*	b*
WBSF	0.211 (0.06)	-0.010 (0.40)	0.073 (0.52)	0.006 (0.96)	0.278 (0.01)	0.332 (<0.01)	0.359 (<0.01)
Lactate	0.267 (0.02)	0.249 (0.03)	-0.209 (0.06)	0.369 (<0.01)	0.042 (0.71)	0.165 (0.15)	0.155 (0.17)
Chute Score		0.179 (0.11)	-0.183 (0.11)	-0.006 (0.96)	-0.192 (0.09)	-0.048 (0.67)	-0.090 (0.43)
Capture Score			-0.385 (<0.01)	-0.005 (0.96)	0.029 (0.80)	0.079 (0.49)	0.091 (0.42)
Exit Velocity				0.136 (0.23)	0.012 (0.91)	-0.036 (0.75)	-0.020 (0.86)

^aCS, CAPS, EV, vocalization, and lactate (n = 126). WBSF, L*, a*, and b* (n = 107).

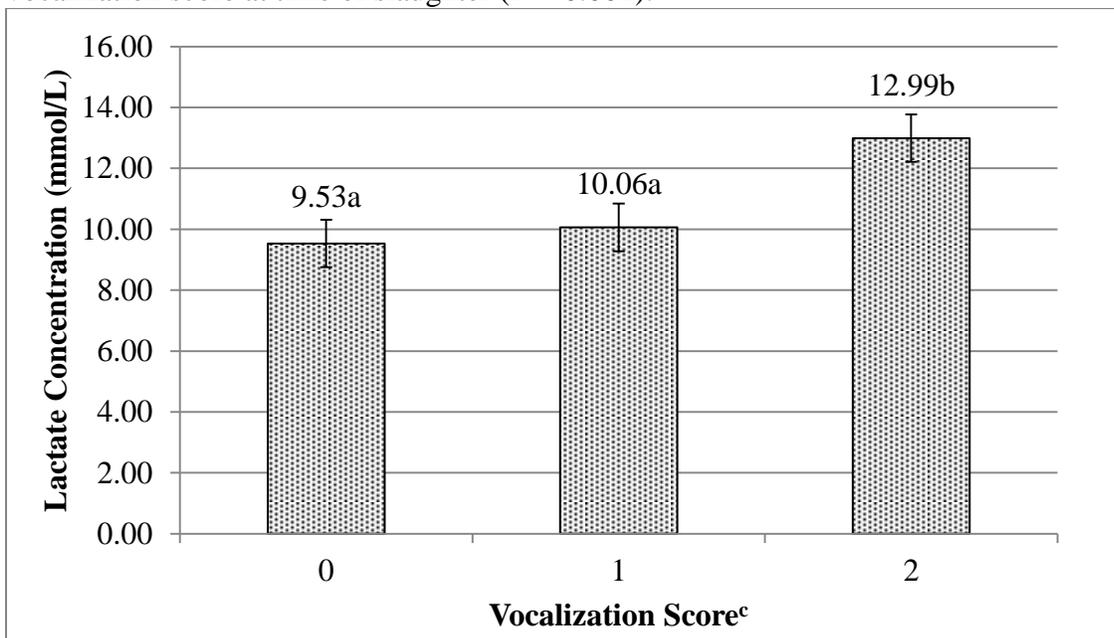
^bL* = electronic color measurement indicating lightness/darkness whereby 100 is pure white and 0 is black, a* = electronic color measurement whereby a positive value is in the red color spectrum and a negative value is in the green color spectrum, and b* = electronic color measurement whereby a positive value is in the yellow color spectrum and a negative value is in the blue spectrum.

Increasing blood LAC levels are associated with increased stress in cattle (Mitchell et al., 1988; Voisinet et al., 1997). Mitchell et al. (1988) reported that plasma lactate spikes are observed as a result of both handling and transport stress, as a result of the hypothalamic-adrenal cortex phase and the sympathetic-adrenal medulla phase. The authors also reported that stunning results in a massive sympathetic response resulting in elevated plasma lactate levels.

Qvisth et al. (2008) reported a similar purge of lactate from the muscle during periods of stress, but noted that adipose tissue is also a significant source of lactate release. The increased

presence of lactate in adipose tissue results in a spike in lipolysis to aid in energy availability to the muscle. In order for lipolysis to occur an aerobic environment is needed (Romijn et al., 1993), and therefore stress immediately preceding harvest may not directly affect carcass composition. A possible mechanism could be elucidated to relate the effects of pre-slaughter stress with a decrease in carcass quality grade. Research attempting to understand this metabolic timeline is needed.

Figure 3.1. Relationship between average blood lactate concentration (mmol/L) and vocalization score at time of slaughter ($P = 0.001$).



^{a, b} Means with different superscripts were different ($P < 0.05$).

^c 0 = no vocalization, 1 = very little vocalization, 2 = excessive vocalization.

Current data collected combined with previously discussed work would imply that environmental stressors through the stunning phase at the packing plant can be measured through blood plasma profiles. Cattle with increased VOCAL scores were also squirming and fighting the v-belt restrainer the most, resulting in a longer duration of time to restrain them prior to captive bolt stunning or exsanguination. It is important to note these differences in head restraint between the two slaughter methods. Cattle stunned with the captive bolt method have their nose

positioned downward, while the Kosher method requires their nose to be elevated to expose the neck. This manipulation in head position alone could be an added stressor just prior to harvest.

Exit velocity and CAPS were negatively correlated ($P < 0.001$; $r = -0.385$), suggesting heifers displaying a more temperamental response to being restrained exited the working chute at a faster rate. These data also show a relationship between feedlot behavior and stress in the plant prior to slaughter. Measurements of temperament correlated with LAC (CS, $P = 0.02$; $r = 0.267$; CAPS, $P = 0.03$; $r = 0.249$; EV, $P = 0.06$; $r = -0.209$). Similar results have been reported by Gruber et al. (2006), reporting that cattle exhibiting restless or agitated behavior at the slaughterhouse following transportation had higher blood lactate concentrations, and produced tougher meat.

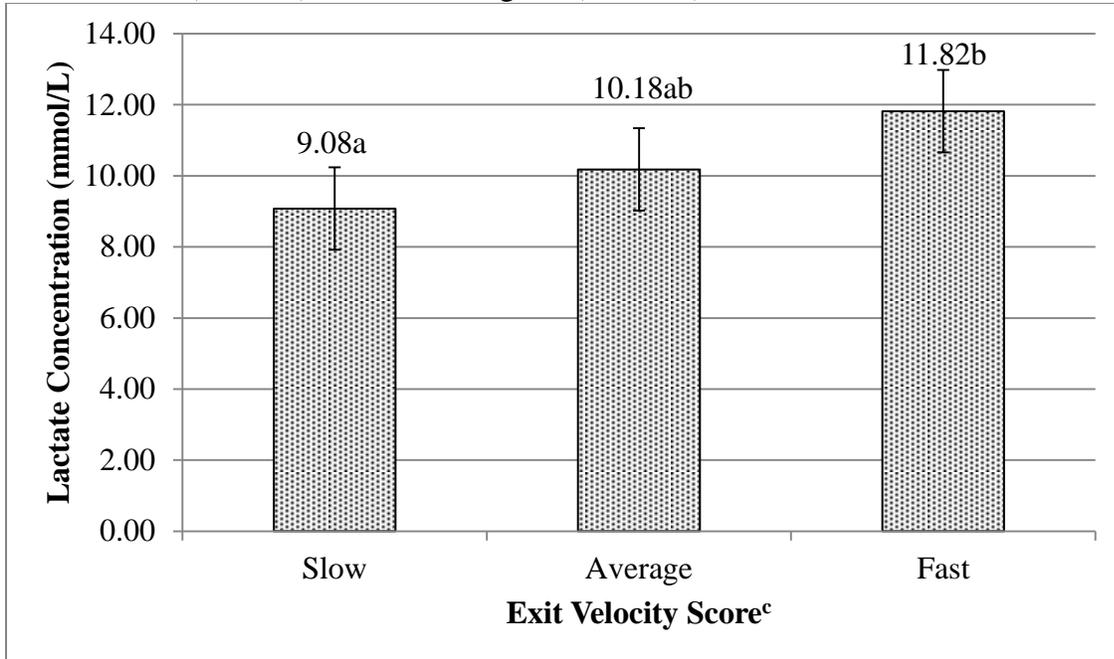
This relationship between EV and LAC is further demonstrated in Figure 3.2. Exit velocity scores were characterized into 3 groups. The “average” cattle included all cattle within 1 standard deviation of the mean EV (1.95 ± 0.77 m/s), while “slow” cattle ($n = 19$) had an EV < 1.18 m/s, and the “fast” cattle ($n = 11$) had an EV > 2.75 m/s. These data further strengthen the understanding and relationship between LAC at time of slaughter with measurements of beef cattle temperament and stress.

Warner- Bratzler Shear Force and Minolta Color Scores

Table 3.1 presents a similar relationship between WBSF and Minolta color scores as reported by Wulf et al. (1997). WBSF was correlated ($P \leq 0.01$) with L*, a*, and b* values ($r = 0.278$, $r = 0.332$, and $r = 0.359$, respectively). Color score may be a potential predictor of beef palatability and tenderness that could be obtained at line speed in a packing plant. Wulf and Page (2000) proposed such a grading system using maturity, marbling, Minolta color scores, and hump height, in an attempt to reduce palatability variations within quality grade. Both proposed

methods proved successful in reducing the palatability variation within quality grade, but unfortunately have yet to be implemented. These alternative methods of quality grading may be more applicable to the development of branded beef programs where marketing can focus on a “guaranteed tender” product.

Figure 3.2. Relationship between exit velocity score in the feedlot and average blood lactate concentration (mmol/L) at time of slaughter ($P = 0.15$).



^{a, b} Means with different superscripts were different ($P < 0.05$).

^c Slow = exit velocity < 1.18 m/s ($n = 19$), Average = 1.95 ± 0.77 m/s ($n = 92$), Fast = exit velocity > 2.72 m/s ($n = 11$).

As discussed previously, Wulf et al. (1997) reported that meat color could serve as an indicator of beef tenderness reporting higher L^* , a^* , and b^* (lighter, redder, and yellower) color scores were associated with more tender beef. Our data is contrary to those reports as meat toughness was positively correlated ($P \leq 0.01$) to lighter, redder, and yellower meat color ($r = 0.278$, $r = 0.332$, and $r = 0.359$, respectively). This relationship is presented in Table 3.2 with Kosher slaughtered cattle possessing higher L^* , a^* , and b^* values ($P \leq 0.001$) compared to the captive bolt stunned cattle (42.77, 27.72, and 8.55 vs. 41.14, 26.24, 7.29, respectively). This inverse relationship may be a result of the Kosher slaughter method. Changes in the state of

muscle contraction and pre-rigor metabolism are both potential explanations for this result. Currently there is no published research evaluating Kosher slaughter and meat quality, and therefore this effect can only be hypothesized.

Table 3.2. Means and standard errors (SE) between slaughter method, slaughter day, and pen for L*, a*, b* Minolta color scores, Warner-Bratzler shear force (WBSF), cook loss, and marbling.

Item	Day 1		Day 2	Method x Day	Pen (Day) P-value	Overall P-value
	Kosher	Captive Bolt	Captive Bolt			
n evaluated	40	6	61			
Color ^a						
L*	42.77 (0.54)	39.99 (1.70)	41.23 (0.45)	0.135	0.129	0.001
a*	27.72 (0.44)	26.18 (1.36)	26.25 (0.36)	0.299	0.389	0.001
b*	8.55 (0.34)	7.33 (1.06)	7.29 (0.28)	0.291	0.176	< 0.001
WBSF (kg)	3.64 (0.15)	3.94 (0.48)	3.19 (0.13)	0.566	0.047	< 0.001
Cook loss (%) ^b	23.0 (1.0)	27.0 (3.0)	26.0 (1.0)	0.268	0.376	0.076
Marbling ^c	387.0 (10.0)	418.0 (32.0)	417.0 (8.0)	0.378	0.232	0.040

^aL* = electronic color measurement indicating lightness/darkness whereby 100 is pure white and 0 is black, a* = electronic color measurement whereby a positive value is in the red color spectrum and a negative value is in the green color spectrum, and b* = electronic color measurement whereby a positive value is in the yellow color spectrum and a negative value is in the blue spectrum.

^bCook loss is the % change in steak weight as the result of cooking to 71°C (160°F).

^cMarbling score designation: 100 = traces (standard), 200 = slight (select), 300 = small (low choice), 400 = modest (average choice), and 500 = moderate (high choice).

Kosher slaughtered cattle typically remain conscious for 17 to 85 seconds after a proper throat cut (Grandin, 2010b) compared to immediate unconsciousness with captive bolt stunning. Data collected by Hayes (2012) suggests some cattle harvested by the Kosher method remain conscious for up to 200 seconds following the throat cut. During this time the muscles are in tense contraction prior to reaching unconsciousness, shortening the muscle fiber length at rigor. This results in more muscle fiber overlap leading to a less tender steak, especially in the *longissimus* complex (Locker, 1959). Based on slaughter floor observation, there is much more visual muscle contraction and reaction following a throat cut than a captive bolt stun. Some of this difference in muscle activity may be a result of the anatomical location of the vertebral arteries. Even with proper severing of these arteries in the neck, the arteries to the brain remain intact, allowing for a prolonged blood supply to the brain (Grandin, 2011). The exacerbated

muscle contraction and tension of the animal prior to reaching unconsciousness could have negative effects that last well beyond the slaughter floor. To reduce this prolonged activity, some slaughter facilities have elected to follow the throat cut with a captive bolt stun to reduce animal activity and blood splash (Grandin, 2010a; Hui, 2012). This would concur with pork data published by Judge et al. (1967), showing that prolonged periods of restraint elicited negative changes in muscle dynamics and was amplified as restraint time increased. The acceptance of a captive-bolt stun following the initial throat cut could potentially alleviate much of the observed animal stress, resulting in a more palatable product. While palatability may not be a primary concern with Kosher beef consumers, it is important to note that retail product palatability should still be a focus when discussing Kosher slaughter. The hindquarters of all Kosher carcasses are sold in the conventional market due to the muscles association with the siatic nerve (Hui, 2012). Forequarters not passing final inspection are also sold through the conventional market.

Marbling and Cook Loss

Table 3.2 includes the marbling scores reported by slaughter method. Strip steaks from captive bolt stunned heifers had increased marbling ($P = 0.04$) compared to the Kosher slaughtered heifers (modest 10 vs. small 80; average choice vs. low choice, respectively). Animals were shipped by pen to maintain feedlot experimental units with the heaviest pens marketed the first week followed by the remaining pens the following week. Current data reveals cattle slaughtered the first week by the Kosher method had lower average marbling scores than cattle slaughtered the second week, with no significant difference in carcass weight. This is contrary to expectation, as faster maturing cattle were marketed first resulting in a higher average quality grade. Another explanation for this difference in marbling score could be the result of variation by the grader. Carcasses were not graded by a computerized system and

therefore week to week variation is possible; especially with the known difference in L^* values between the two treatments. The Kosher slaughter animals possessed a higher ($P \leq 0.001$) L^* value potentially making marbling less apparent. These data are opposite to reports by Breidenstein et al. (1968) who reported steaks with a higher marbling score had a significantly higher color score. With the current void in Kosher slaughter meat quality research this relationship between marbling and color is difficult to traverse.

The observed relationship between color and marbling goes against traditional meat science understanding, and hence challenges us to develop an alternative theory. Is there an alternative reaction taking place in the conversion of muscle to meat utilizing adipose tissue as an energy source during this period of increased stress at time of slaughter? Could this be exacerbated by the fact the animal is conscious for a longer period of time? Published data would suggest 200 s is an insufficient time to cause a measurable effect. Qvisth et al. (2008) noted the increased purge in plasma lactate in stressed cattle which is known to increase the rate of lipolysis to meet energy demand. The previously discussed timeline may or may not allow adequate time between pre-harvest and rigor to influence intramuscular fat depots. In either case, the question persists of how long it takes cattle to utilize adipose tissue to an extent where differences can be measured through marbling score.

Strip steaks from captive bolt stunned heifers had a tendency ($P = 0.08$) for increased cook loss compared with kosher slaughtered heifers. This relationship is also inverted from traditional understanding. Gault (1985) reported that higher L^* values are representative of a lower final pH and an increased cook loss. Carcass drip loss measurements would have been beneficial to see if there was a shift in water-holding capacity across treatments. Mitsumoto et al. (1995) described results where steaks had a reduced drip loss but subsequent cook losses were

amplified. The authors credited improved cell integrity for this difference. Warner et al. (2007) reported cattle induced with a 15 m stressor had a 1.9 % reduction in water-holding capacity compared to controls (3.5 % vs. 5.4 %). Kosher slaughtered carcasses may have had an increased drip loss, reducing the available free water in the muscle, leading to a reduced cook loss compared to the captive bolt stunned heifers.

One confounding factor to the cook loss data could be the difference in marbling score. Akinwummi et al. (1993) reported that cook losses tend to increase with increasing marbling. The observed difference in marbling score and cook loss suggests there are changes in postmortem metabolism and muscle ultrastructure as a result of slaughter differences.

Implications

There is a positive relationship between feedlot temperament measurements and blood lactate concentration at time of slaughter, suggesting that an animal's stress response in the feedlot is similar to their response at the slaughterhouse. Slaughter method can have an impact on meat quality and palatability, as strip steaks from Kosher slaughter heifers had increased L* values, decreased WBSF values, reduced marbling scores, and reduced cook loss. An in depth investigation of the changes in muscle structure and overall meat quality as a result of the Kosher method is needed to understand the implications of this alternative slaughter method.

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