

PHENOTYPIC AND GENETIC EFFECTS OF DISPOSITION  
ON BEEF TENDERNESS AND QUALITY ATTRIBUTES

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by  
TASIA MARIE TAXIS

Drs. Jeremy F. Taylor and Robert L. Weaber,  
Thesis Advisors

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The undersigned, appointed by the Dean of the Graduate School, have  
examined the thesis entitled

PHENOTYPIC AND GENETIC EFFECTS OF DISPOSITION  
ON BEEF TENDERNESS AND QUALITY ATTRIBUTES

Presented by Tasia Marie Taxis

A candidate for the degree of Master of Science

And hereby certify that in their opinion it is worthy of acceptance.

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Dr. Robert L. Weaber

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Dr. Jeremy F. Taylor

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Dr. Robert D. Schnabel

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Dr. Mark R. Ellersieck

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# DEDICATION

This thesis is dedicated to my family and friends who have provided me with encouragement throughout the completion of my degree. My parents, Craig and Mary Ann Taxis have instilled in me the values of hard work and responsibility, and always encouraged me to pursue my dreams; my brother, Travis Taxis for his inspiration, persistence, and understanding; Liz Kolb and Chad Stauffer for their friendship, listening ears, and continuous support; and Drs. Eduardo Casas and Chris Bidwell for pushing me towards a Master's degree. I could not have accomplished my goals without your continuous support. All of you have played pivotal roles in making me the person I am today and in helping me reach this important stepping stone in life – for that I will forever be grateful.

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## ABBREVIATIONS

AAA	American Angus Association
ADG	Average Daily Gain
AMSA	American Meat Science Association
APSF	Average Peak Shear Force
ASA	American Simmental Association
BIC	Bayesian Information Criteria
BIF	Beef Improvement Federation
BIGS	Bioinformatics to Implement Genomic Selection
CG	Contemporary Group
EPD	Estimated Progeny Difference
EV	Exit Velocity
FT	Flight Time
G-BLUP	Genomic Best Linear Unbiased Prediction
GWAS	Genome-Wide Association Study
LD	<i>Longissimus dorsi</i>
LM	Longissimus muscle
MBV	Molecular Breeding Value

NALF	North American Limousin Foundation
QTL	Quantitative Trait Loci
SNP	Single-Nucleotide Polymorphism
WBSF	Warner-Bratzler Shear Force

# PHENOTYPIC AND GENETIC EFFECTS OF DISPOSITION ON BEEF TENDERNESS AND QUALITY ATTRIBUTES

Tasia Marie Taxis

Drs. Jeremy F. Taylor and Robert L. Weaber

## ABSTRACT

Cattle temperament (exit velocity; EV) and steak tenderness (Warner-Bratzler shear force) have been shown to be associated in *Bos indicus* cattle (Behrends et al., 2009). Both traits potentially provide opportunities for improvement among beef herds and are profitable to producers. The American Simmental Association (ASA) provided records which included pedigree information, multiple WBSF core values, reported as average peak shear force (APSF), and a maximum of two EV measurements taken 42 days apart. Bayesian Information Criteria (Gilmour et al., 2006) values were utilized to evaluate the fit of alternative statistical models to the data. A near zero genetic correlation was estimated between APSF and EV. Moderate heritability estimates were found for both APSF and EV. DNA was extracted from tissue samples and genotyped using the Illumina BovineSNP50 BeadArray (San Diego, CA, USA; Matikumalli et al., 2009). Genome-wide association studies were conducted to identify genomic regions harboring loci associated with either of the traits. Only 70 (0.167%) of the 42,351 tested SNP markers were associated with variation in APSF ( $n = 957$ ), and 2 (0.006%) of the SNP markers were associated with variation in EV ( $n = 599$ ). The difference between pairs of

EV measurements ( $n = 587$ ) was also analyzed as a measure of habituation to human handling, and 2 (0.006%) of the SNP markers were found to be associated.

# CHAPTER 1

## LITERATURE REVIEW

### Introduction

Steak tenderness and cattle temperament are two traits that when improved among a beef herd can be profitable to the producer. Standardization of both traits has been studied since the 1900s. In the late 1920s, Warner-Bratzler shear force became the method to measure beef tenderness, and is the most widely used method in beef research today. The trait is moderately heritable, which provides the opportunity for selection for tenderness. With the advent of SNP Chip technology, two main candidate genes,  $\mu$ -calpain (*CAPNI*) and calpastatin (*CAST*), have been found to be associated with tenderness, and some breed associations have published molecular breeding values (MVB) for this trait. Temperament became of interest to breed associations in the 1980s and researchers are still establishing a standardized measurement protocol. Exit velocity is the only objective measurement used to date, and therefore has been highly used in research studies. Temperament has been a particular focus in *Bos indicus* cattle, and has been shown to be moderately heritable. Correlations between exit velocity and other production traits have been found, including tenderness. Chapter 2 examines the genetic and phenotypic correlations among these traits as well as the heritability of Warner-

Bratzler shear force and exit velocity in *Bos taurus* cattle, specifically Angus, Simmental, or Angus  $\times$  Simmental crossed breeds. Chapter 3 completed a genome-wide association study to detect single-nucleotide polymorphism markers associated with temperament or tenderness in Simmental  $\times$  Angus crossbred cattle.



## **Tenderness**

In the late 1920s K. F. Warner and his associates first established the idea of shearing a piece of cooked meat to produce an indication of tenderness (Wheeler et al., 1997). After the Warner Bratzler shear force (WBSF) apparatus was invented, blade thickness, sharpness, and the size and shape of the hole in the shear blade were perfected by Bratzler (Wheeler et al., 1996). To date, WBSF is the most widely used instrumental measure of meat tenderness (Wheeler et al., 1997). However, each institution uses varying protocols for WBSF measurements. The diversity in protocols makes it virtually impossible to directly compare shear force values among data published by different institutions. An important recommendation from the National Beef Tenderness Conference (1994) was to standardize a WBSF measurement protocol. For producers as well as industry to provide tender beef, an understanding of the factors that influence tenderness need to be accurately defined and measured across institutions. Most recently, research focus has been placed on the cooking and coring factors affecting the WBSF measurements.

The cooking process has been scrutinized because when considering a set protocol, thawing conditions, cooking method, cooking rate, and degree of doneness all affect steak tenderness (Wheeler et al., 1997). Steaks are often aged and then frozen in research, while in industry practices steaks are usually never frozen. Some research shows that freezing steaks after they've been aged results in a lower WBSF (Law et al., 1967), however, conclusions from other research are varied. Pearson and Miller (1950) found detrimental effects on tenderness of freezing steaks, while others have found no

difference in WBSF values between frozen and fresh steaks (Smith et al., 1969; Obuz and Dikeman, 2003). While all these studies are similar in many aspects, they utilized varying postmortem aging intervals, and the length of postmortem aging affects WBSF values. The average aging of fresh beef at retail in the United States is approximately 18 to 22 days (Shanks et al., 2002). Therefore, most researchers utilize a 14 to 21 day aging period to simulate industry conditions (Shanks et al., 2002). The research that has compared frozen to fresh steaks in this postmortem aging interval has found no difference in WBSF values.

Another factor that needs to be standardized in the WBSF protocol is the method of cooking. Each shear force machine produces a different consistency and repeatability measure. Machinery used to cook the steaks includes open hearth electric broilers, tabletop convection broil ovens, belt grills, and forced-air convection ovens. The open hearth electric broilers and convection ovens have reduced repeatability and inconsistent cooking (Wheeler et al., 1997; Lawrence et al., 2001). It seems that the belt grills are consistent and the repeatability of WBSF measurements produced by these cooking instruments is higher than for the other machinery. The belt grill runs the steaks on Teflon-coated conveyor belts between electrically-heated metal plates. When the grill is set to 163°C, the repeatability is significantly improved (Wheeler et al., 1997; Lawrence et al., 2001). A less expensive option to the belt grill is a clam-shell grill. This machine has acceptable repeatability and is considered a viable WBSF cookery method (Yancey et al., 2011).

End-point temperature is yet another cooking factor that affects WBSF measurements. It's been shown that higher end point cooking temperatures result in higher shear force measurements (Wheeler et al., 1999; Yancey et al., 2011). It is important that the end point temperature used mimics the consumer's cooking preference. Since each consumer's preference varies, this may be the most challenging factor to standardize.

In the last few years, researchers have focused on this by comparing WBSF measurements to trained sensory panel ratings. Schmidt et al. (2010) found that consumers preferred the texture of rare and medium rare steaks with end point temperatures of 60°C and 66°C, respectively. Warner-Bratzler shear force values were comparable between steaks cooked to 60°C, 71°C, and 74°C (Schmidt et al., 2010). Wheeler et al. (1997) suggested grouping steaks into three groups (WBSF < 3.0-kg, 3.0 to 5.7-kg, or > 5.7-kg) based upon consumer's acceptance level; the lowest WBSF group is 100% acceptable while the highest WBSF is 100% unacceptable to consumers. However, drawing conclusions between a subjective and objective measure will pose a challenge in standardizing end-point temperature.

Although now better standardized, coring has also been a factor of concern for WBSF measurements. Core location and orientation have both been shown to affect WBSF measurements. Kerth et al. (2002) found a lateral to medial tenderness gradient across steaks. However, Wheeler et al. (1996) found no such gradient. Whichever the case, when multiple cores are taken from a random dispersal over the entire steak, a maximum of six cores sheared from each steak is all that is needed for the best

repeatability (Wheeler et al., 1996). When cores are removed parallel to the fiber orientation, the WBSF value is greater than that of cores taken perpendicular to the steak surface (Murray et al., 1983; Wheeler et al., 1994). The cores producing the most accurate and repeatable tenderness measures are those sheared parallel to the long axis of the muscle fibers (Wheeler et al., 1997). Other factors investigated in obtaining cores include the difference between hand and machine sampled cores, carcass maturity, and fiber diameter (Tuma et al., 1962; Francis et al., 1981; Wheeler et al., 1994).

It is important that the protocol for WBSF measurement be standardized but also be utilized across all institutions. In the 2005 National Beef Quality Audit, inadequate beef tenderness was ranked the second top quality challenge or concern of beef producers (including seedstock producers, cow-calf producers, stocker/backgrounders, and feedlot operators). Both stockers/backgrounders and feedlot operators indicated that they tried various practices to improve this challenge, one of which was to collect and use carcass data (Shook et al., 2008). A protocol for WBSF needs to be documented across all institutions to ensure accurate, precise, and comparable data from all research institutions or progress cannot be made.

The American Meat Science Association (AMSA) in its 1995 guidelines for cookery, sensory, and tenderness recommended some guidelines for WBSF. Beef samples are suggested to be cut 2.54-cm thick, vacuum packaged and aged for 14 days. When cooking steaks, AMSA does not recommend using an air convection oven, but if using a belt grill to set it at 163°C (Lawrence et al., 2001; Yancey et al., 2011). The steaks should be cooked to a 71°C internal temperature (Wheeler et al., 1997). After the

cooked steaks are chilled 24 hours at 3°C, six 1.27-cm diameter cores that represent the entire steak should be removed parallel to the muscle fibers (Wheeler et al. 1997; Otremba et al., 1999; Wheeler et al., 1999). The AMSA suggests that coring through the center of the shears using a calibrated Universal Testing Machine with a Warner-Bratzler shear attachment (V-notch blade) at 200 to 250-mm/min crosshead speed (Wheeler et al., 1997; Wheeler et al., 1999; Obuz and Dikeman, 2003). The protocol used for all WBSF measurements in the following studies followed the protocol outlined by Dikeman et al. (2005), which closely follows all of the AMSA recommendations as well as the proposed shear force procedures for meat tenderness measurements (Wheeler et al., 2005).

Since beef packers, restaurateurs, and retailers rank tenderness as one of the highest beef quality concerns, it is beneficial for producers to produce a consistently tender product (Lusk et al., 2001). Platter et al. (2005) concluded that as shear force increased, the probability of the consumers buying the steak greatly decreased. Consumers were willing to pay an average of \$7.20 for a WBSF value < 3.40-kg, whereas the consumer would pay approximately \$3.00 less for a steak with a WBSF value > 5.40-kg. If together, scientists and producers could improve product consistency and eating quality, a higher demand for the product would arise. Therefore, scientists should feel compelled to concentrate on not only the details and factors of WBSF measurements, but also the genetic variation and heritability of the trait.

WBSF measurements vary across breeds, but more so between *Bos taurus* and *Bos indicus* cattle. As the amount of *Bos indicus* breed increases in the individual animal, the less tender is the meat. In other words, the higher the influence of *Bos taurus* breeding

in an animal, the more tender is the steak (Crouse et al., 1989; Miller et al., 1996). Johnston et al. (2001) found that the average measure of WBSF was 6.93-kg with heritability of 0.19 across a few *Bos indicus* breeds (Belmont Red, Brahman, and Santa Gertrudis). These results were similar to other papers showing Brahman with an average WBSF measurement of 7.76-kg (Burrow et al., 2001). Crouse et al. (1989) found that Brahman had an average WBSF measurement of 5.88-kg. *Bos taurus* WBSF measurements range from 4.41 to 5.62-kg. This includes the Hereford (4.40-kg), Angus (4.41-kg), Limousin (5.62-kg), Simmental (5.49-kg), Piedmontese (5.40-kg), and Charolais (5.17-kg) cattle breeds (Crouse et al., 1989; Burrow et al., 2001).

Heritabilities found by Minick et al. (2004) were Angus (0.33), Simmental (0.16), Hereford (0.11), and Charolais (0.46). Aass et al. (2010) found the heritability of tenderness in Norwegian Red cattle to be 0.23. While it is evident that WBSF values are higher in *Bos indicus* breeds of cattle, the heritability estimates vary in both *Bos indicus* and *Bos taurus*. Within *Bos taurus* cattle, Angus appears to be more tender than Limousin, Gelbvieh, Simmental, and Charolais (Page et al., 2004).

Estimates suggest that 12 to 43% of the variation in beef tenderness is due to additive genetics (Minick et al., 2004). Due to the moderate heritability of beef tenderness and the goal for better productivity, the Carcass Merit Program was initiated. The primary objective of the program was to genetically identify superior animals in the United States beef cattle population that would produce progeny with the greatest potential for meeting consumer demands. Warner-Bratzler shear force was included among carcass trait measures in hopes of identifying genetic markers associated with beef

tenderness between breeds. At the 54<sup>th</sup> Annual Reciprocal Meat Conference in 2001, the project had identified 6 quantitative trait loci (QTL) that were segregating between breeds for WBSF measurements. The U.S. Meat Animal Research Center has also identified two loci that effect the WBSF measurement of the longissimus muscle (LM) (Smith et al., 2000). Currently, the gene *CAPNI*, which encodes for  $\mu$ -calpain, and its inhibitor calpastatin (*CAST*) are the two principle candidate genes identified with 14 day postmortem tenderness in beef (Casas et al., 2003; Page et al., 2004; Casas et al., 2006).

Tenderness of beef is dependent on the proteolytic breakdown of muscle during the postmortem interval. *CAPNI*, found in the central region of BTA29, is orthologous to regions on HSA11 (Smith et al., 2000), and has been termed the most important enzyme in beef tenderness (White et al., 2005). *CAPNI* is an intracellular cysteine protease, and codes for the enzyme  $\mu$ -calpain.  $\mu$ -calpain is a calcium-activated protease which is activated with micromolar concentrations of  $\text{Ca}^{2+}$  in the early postmortem period. When calcium binds to  $\mu$ -calpain, the enzyme becomes active. This active state enables  $\mu$ -calpain to breakdown the myofibrillar structures in muscle, ultimately causing the muscle/steak to become more tender (Koohmaraie, 1996; Kemp et al., 2010). Calpastatin is also responsible for the tenderness of beef (Zhou and Hickford, 2008; Kemp et al., 2010). Calpastatin is encoded by the *CAST* gene, which is located on BTA7 (Schenkel et al., 2006). Calpastatin is an unstructured protein until it binds to an active state  $\mu$ -calpain molecule. Once bound, it adopts a structure which inhibits  $\mu$ -calpain activity. Higher levels of calpastatin lead to reduced activity of  $\mu$ -calpain, therefore reducing proteolysis which is required for tender meat (Camou et al., 2007; Kemp et al., 2010). Marker-

assisted selection for beef tenderness became possible when single-nucleotide polymorphisms (SNP) within the *CAPNI* and *CAST* genes were associated with WBSF. For marker-assisted selection to produce the greatest impact in the cattle industry, marker panels need to be useful across breeds. Two SNP markers have been utilized in the *CAPNI* gene, *CAPNI-316* and *CAPNI-530*. A homozygous *CC* animal at *CAPNI-316* and a *GG* animal at *CAPNI-530* have been shown to have lower WBSF measurements of 0.30-kg and 0.20-kg, respectively, in all seven of the most popular *Bos taurus* breeds in the United States (Page et al., 2004; Corva et al., 2007; Café et al., 2010). However, variation in *CAPNI-530* is rare among British breeds (Corva et al., 2007). Currently, GeneSTAR (Pfizer Genetics Ltd.) and Igenity (Merial Ltd.) are two genomic companies that provide tests that associate markers within *CAST* and *CAPNI* genes with beef tenderness for producers to implement marker-assisted selection (Zhou and Hickford, 2008; Kemp et al., 2010). The GeneSTAR Tenderness 2 (Bovigen LLC, [www.bovigen.com](http://www.bovigen.com)) test uses a marker *CAST-T1* for the calpastatin gene, and the two previously described markers for the  $\mu$ -calpain gene (Van Eenennaam et al., 2007; Zhou and Hickford, 2008). The substitution of a *T* allele in the *CAST-T1* marker was associated with a decrease of 0.15-kg in WBSF measurements. When a *C* allele was substituted in both the  $\mu$ -calpain marker locations a decrease of 0.34-kg in WBSF was found (Van Eenennaam et al., 2007; Johnston and Graser, 2010). The Igenity *TenderGENE* (Merial, <http://www.igenity.com>) test produced similar results. Igenity uses one *CAST* marker (*G/C* SNP in intron 5) to determine variability in WBSF (Zhou and Hickford, 2008). The company also uses two  $\mu$ -calpain markers, *CAPNI-316* and *CAPNI-4751*. Tenderness



improved 0.19-kg with each increase of the *C* allele in the calpastatin SNP markers (Schenkel et al., 2006; Van Eenennaam et al., 2007). When there was an increase in of one *G* allele at in the *CAPNI-316* gene and one *C* allele at the marker *CAPNI-4751*, WBSF measurements were decreased by 0.33-kg. In both tests, *CAPNI* has a greater effect than *CAST* on tenderness. The *CAPNI-316/4751 C/C* haplotype is associated with the lowest WBSF and the *C/T* haplotype is rarely seen in both *Bos taurus* and *Bos indicus* breeds (Van Eenennaam et al., 2007; Café et al., 2010). *CAPNI* has a greater influence on WBSF measurements and a high *G/T* haplotype frequency (> 0.50) present in the US populations. The beef industry has the opportunity to make improvements in tenderness by selecting for the *C/C* haplotype. It is also important to note that both companies used *Bos taurus* or *Bos indicus* × *Bos taurus* cross populations for validation, and the alleles were variable at all loci. This variation is not found in purebred *Bos indicus* populations (Van Eenennaam et al., 2007). Because allele frequencies are variable in different breeds, one genetic test to improve WBSF measurements may not work across all breeds of cattle.

When results from the SNP panels are combined and the net genetic effects are estimated across loci, they are referred to as molecular breeding values (MBV). Weaber and Lusk (2010) estimated the possible value of genetic improvement and revenue from utilizing MBVs in selection decisions in beef herds. By selecting the top 10% of MBV ranked bulls with heifer replacement based on MBV, WBSF was reduced by 7.2% and \$11.3 billion in national benefits were projected in 20 years. A less aggressive selection approach of selecting bulls with the top 50% of MBVs and without heifer replacement

using MBVs, WBSF was reduced by 2.5% and projected \$5.3 billion in national benefits by year 20. The projected benefits were distributed to consumers (31%), the retailers (10%), the packing sector (3%), the feedlot sector (7%), and to all other firms involved in supplying feeder cattle (49%). As mentioned earlier, genetic improvement of WBSF is difficult because beef tenderness is a complex trait affected by many factors, both environmental and genetic (Minick et al., 2004; Weaber and Lusk, 2010). The phenotypic data are difficult and expensive to collect. To measure WBSF, cattle must be followed to the packing plant for collection of steak samples. Following collection, each steak must be aged, cooked, and then sheared. Also, the packing industry is reluctant to remove these samples, because it significantly degrades the total value of the product (Weaber and Lusk, 2010). Most SNP markers are associative rather than causative (Weaber and Lusk, 2010). However, when a MBV is used in combination with an expected progeny difference, accuracy of genetic predictors is enhanced. . Utilizing these technologies provides the best estimates of each animal's genetic merit, and will lead to faster herd improvement.

## Temperament

According to Burrow (1997), temperament is defined as an animal's behavioral response to human handling. Aggressive cattle pose problems to the humans who are handling them, as well to farm equipment and the animal's own safety. Calmer temperament animals adapt more easily and become less stressed with repeated handling while the more excitable animals have a greater difficulty adapting to repeated handling procedures (Grandin, 1997). Temperament in beef cattle is associated with performance, health, and carcass quality traits (Café et al., 2011; Curley et al., 2006; Nkrumah et al., 2007). Since temperament is measured early in an animal's life, and is associated with other production traits, disposition measurements are an economically relevant trait that should be considered by beef producers when breeding or purchasing cattle (Beckman et al., 2007).

There are many proposed subjective and objective methods for measuring temperament in cattle. The subjective measurements include crush test, chute score, and pen score. The crush test ranks the overall temperament of an animal via its movements while entering and individually confined in a crush, or working chute (Burrow, 2003; Kilgour et al., 2006). The willingness of the animal to enter the crush is ranked on a 1 (enters without hesitation) to a 4 (strenuous resistance) scale. While confined in the crush for 2 minutes, the animal's movement is scored on a 1 (no movement) to a 7 (struggles violently and attempts to jump out) scale. If an animal bellows, kicks, or kneels, its movement score is increased by 1, and if the animal lies down, the movement score is increased by 2. The total of these scores is the crush score (Kilgour et al., 2006). The

chute score is taken when the animal is confined but not restrained in a working chute (Curley et al., 2006). The score is ranked on a scale of 1 (calm) to 6 (extremely excited). Pen score is a visual assessment of the animal while being confined in a pen, usually with 4 or 5 other animals. Handlers approach the groups of animals, and then record the animal's temperament on a scale of 1 (calm) to 5 (extremely excited). A more in depth description of each scale of temperament for both the chute score and pen score are shown in Table 1.1. The Beef Improvement Federation (BIF) suggests scoring beef cattle temperament while an animal is in a squeeze chute, using a scale of 1 (calm) to 6 (extremely excited). Several breed organizations, such as the American Angus Association (AAA) and the North American Limousin Foundation (NALF) have adopted BIF's recommended method for subjectively scoring temperament. Other subjective measures include the French score, milking temperament, and auction ring behavior (Lewis and Hurnik, 1998; Sapa et al., 2006; Lanier et al., 2000). All three are based on a similar numeric scale of 1 through 4 or 1 through 5. The docility test is a mix of subjective and objective measurements to record an animal's temperament. This test measures locomotion, changes in mobility, and aggressiveness towards humans (Burrow, 1997; Beckman et al., 2007). The animal is placed in a pen and a handler attempts to confine the animal in a corner. The aggressive score is a subjective test which indicates how threatening the animal is toward the handler. The running time, or the time the animal is in motion, and the number of escapes is recorded during the first 30 seconds from the beginning of the test and then in the presence of a motionless handler for another 30 seconds. Then, the handler is to try to confine the animal in a corner of the pen while

trying to touch/stroke the animal. The amount of time the animal is in motion and the escapes per minute is then calculated. These measurements are combined to approximate a normal distribution for the complete docility score (Sapa et al., 2006).

Subjective measures of temperament in production situations have been called the “trouble makers.” Not only are they time-consuming and difficult to implement, subjective measurements are always subject to human error or bias (Curley et al., 2006). Also, the crush test and chute score may not accurately reflect an animal’s behavior while not in the crush or chute. Some cattle demonstrate a freeze response when restrained, and may appear to have a calmer temperament score than they should receive (Burrow and Corbet, 2000; Burrow, 2003). After observing that animals remain calm while being weighed but leave the weigh scale at different speeds, Burrow et al. (1988) proposed the use of exit velocity (EV) as an objective measure of temperament in cattle. Exit velocity is the rate (m/s) at which animals exit the working chute and covers the distance of 1.7-m (Burrow et al., 1988; Curley et al., 2006). Two light beams are focused on infra-red sensors spaced at the chute (head bail) and 1.7-m away from the head bail. The sensors have an on/off mechanism, so as the animal breaks the light beam, the timing apparatus stops and a connected computer records the time (Burrow et al., 1988; Curley et al., 2006; Beckman et al., 2007). The time it takes the animal to pass between the two sets of infra-red sensors is recorded and called flight time (FT). Flight time is recorded in hundredths of a second and converted into a velocity (m/s) termed EV. Consequently, the poorer the temperament of an individual animal, the higher the EV value (Beckman et al., 2007). It is important to determine the correct time in the animal’s life to measure EV. At

weaning, there is no difference in EV between the sexes of the animals, but the measurements become significantly different between sexes by 18 months of age (Burrow et al., 1988; Burdick et al., 2009). At weaning, EV differs between individual animals, but by 18 months of age the differences becomes moderately variable (Burrow et al., 1988). It makes sense to measure the EV of animals at weaning. Not only do producers take weights at around this age, but the variation between animals is expressed, and isn't influenced by the sex of the animal. It has also been noted that while the EV changes through an animal's life span, it is not significantly different from weaning to later in life (Fell et al., 1999; Behrends et al., 2009).

The different methods to measure temperament, both subjective and objective, have different heritability estimates. The crush test has heritability estimates of 0.03 and 0.46 in *Bos taurus* and *Bos indicus* cattle, respectively (Beckman et al., 2007). Chute scores and docility test heritabilities were estimated to be 0.20 (Nelore), and 0.22 (Limousin), respectively (Carneiro et al., 2006; Beckman et al., 2007). Exit velocity has heritability estimates of 0.40 among *Bos indicus* breeds, 0.36 in the Canadian Beef Cattle Reference herd, and 0.35 among *Bos taurus* breeds (Burrow and Corbet, 2000; Beckman et al., 2007). When EV is taken at weaning, Burrow et al. (1988) reported a heritability of 0.54 in *Bos indicus* breeds. Other studies report similar heritability estimates for temperament using EV; 0.49 in *Bos taurus* breed crosses (Nkrumah et al., 2007) and 0.31 in *Bos taurus* × *Bos indicus* breed crosses (Johnston et al., 2003a; Kadel et al., 2006). Animals with a higher *Bos indicus* content have a higher heritability for temperament (Burrow and Corbet, 2000). Not only is EV the most heritable and repeatable

measurement of temperament in both *Bos indicus* and *Bos taurus* breeds, it has the ability to easily be implemented into a production system (Burrow and Corbet, 2000; Curley et al., 2006; Muller and von Keyserlingk, 2006). Exit velocity appears to be the best available measurement for incorporating temperament into breeding programs (Burrow and Corbet, 2000; Fell et al., 1999).

In 1998, the NALF implemented the first estimated progeny difference (EPD) for docility in beef cattle in their national cattle evaluation. The EPD describes the additional percentage of a sire's progeny that will fall into the calmest chute score (Beckman, 2008; Beckman et al., 2007). According to NALF, there has been a 15% increase in the mean docility EPD over the past twenty years (NALF, 2006). In 2008, the AAA followed NALF's lead and released a docility EPD sire listing in their national cattle evaluation (Beckman, 2008). The American Salers Association also has routine genetic evaluations for docility.

In Holstein cattle, three quantitative trait loci (QTL), on BTA5, BTA18, and BTA29, have been associated with temperament (Hiendleder et al., 2003). The Canadian Beef Cattle reference herd found QTLs associated with temperament on BTA1, BTA5, BTA9, BTA11, BTA14, and BTA15 (Schmutz et al., 2001). Currently, no causal mutations in genes have been identified for temperament in cattle.

While some work has been conducted to implement selection for temperament, it is important when designing cattle breeding programs, to understand the relationship between different traits. Selection to improve one trait may lead to changes (favorable or unfavorable) in another trait. While most of the beef cattle temperament research has

been conducted using *Bos indicus* influenced breeds, conclusions on correlations between traits in *Bos taurus* cattle are likely to be similar.

The correlations between growth, fertility, and carcass quality traits with temperament have been most frequently studied. Poorer temperament animals (animals with higher EVs) spend less time eating (Café et al., 2011), possibly due to a loss of appetite (Carneiro et al., 2006). Café et al. (2011) found that Brahman cattle spent 4.6-min/day less time eating for each m/s increase in EV and a 17.6-min/day decrease in Angus cattle. The decrease in time spent eating could also explain the reduced dry matter intake (370-g for every m/s increase in EV) and feed conversion efficiencies found in poorer temperament animals (Burrow, 2003; Nkrumah et al., 2007; Café et al., 2011). It is also not surprising to see correlations with temperament and average daily gain (ADG) (Voisinet et al., 1997; Fell et al., 1999; Lanier et al., 2000; Burrow, 2003; Carneiro et al., 2006; Muller and von Keyserlingk, 2006; Behrends et al., 2009; Café et al., 2011). A poorer temperament in purebred *Bos indicus* cattle, *Bos indicus* × *Bos taurus* cattle, and *Bos taurus* purebred animals was associated with a decrease of 0.38, 0.19, and 1.46-kg/day in ADG (Voisinet et al., 1997; Fell et al., 1999; Burrow, 2003). Burrow (1997) suggested that selection for high growth rates should improve an animal's temperament. Some studies have shown that animals with a faster EV have elevated physiological concentrations of plasma cortisol (Fell et al., 1999; Curley et al., 2006). A difference of 61.4-nmol/L was seen before weaning and 168.1-nmol/L at feedlot entry in Angus × Hereford cattle (Fell et al., 1999). Elevated cortisol for a short period of time is not detrimental to the long-term health of the animal and may even enhance immune function



(Burdick et al., 2009). However, Beckman et al. (2007) found that animals with poorer temperament had a lower immune function.

The dairy industry has investigated the milk production of nervous or aggressive animals. When dairy cattle are stressed, oxytocin secretion is reduced leading to a 25 to 30% decrease in milk production (Voisinet et al., 1997; Lanier et al., 2000; Curley et al., 2006). Therefore, milk yield and milk flow is decreased (Burrow, 1997; Hiendleder et al., 2003; Muller and von Keyserlingk, 2006). Reproductive traits are affected by the temperament of the animal in artificial insemination programs. Calmer animals (lower EV) cycled more often, were more often visually detected to be in estrus, had an increased number of perceivable estrus, and higher conception rates (Burrow et al., 1988; Carneiro et al., 2006); however, there was no difference in pregnancy rates (Burrow et al., 1988). The conclusion of a higher conception rate but not pregnancy rate could be due to the fact that calmer animals are more tolerant of human contact and are therefore more likely to be inseminated at the appropriate time. Scrotal circumference was also weakly but favorably correlated with exit velocity (Burrow, 2001).

Transporting cattle induces a great amount of stress on the animal. The more docile animals lose less weight during transit and less time is needed for the animal to regain weight after arrival (Burrow, 2003). Greater travel distances, inadequate handling facilities at packing plants, and higher speeds of plant operations all result in greater animal stress resulting in poorer meat quality. Wilder temperament animals have an average of 1.5-kg more bruise trim per carcass due to injuries sustained during transportation (Burrow, 1997; Curley et al., 2006). This results in a negative correlation

between temperament and carcass weight (Nkrumah et al., 2007; Ribeiro et al., 2007; Behrends et al., 2009; Café et al., 2011;). Café et al. (2011) found a 9.9-kg difference in the carcass weight of Brahman cattle with each m/s increase in EV. Ribeiro et al. (2007) reported that steers with a calmer temperament had a 9.2% heavier hot carcass weight. As well as having heavier carcass weights, calmer animals also had larger ultrasound longissimus muscle (LM) and carcass LM areas (Nkrumah et al., 2007). A favorable correlation was reported between temperament and carcass quality measured as yield grade, dressing percentages, and carcass marbling score (Burrow, 2003; Nkrumah et al., 2007; Ribeiro et al., 2007). Alteration of steak taste and eating quality is also associated with temperament. Pre-slaughter stress can deplete muscle glycogen, which results in the meat having a higher pH (Burrow, 1997; Carneiro et al., 2006; Café et al., 2011). Beckman et al. (2007) concluded that docile calves returned \$62.19 per head more than did temperamental calves when sold as beef.

In tropically adapted beef breeds a strong correlation exists between temperament and steak tenderness. Studies have found that the genetic correlation between temperament, measured as FT, and tenderness measured as Warner-Bratzler shear force (WBSF) was -0.42 with FT taken post-weaning and -0.32 with FT taken at the start of finishing (Kadel et al., 2006). These results are similar to those of Behrends et al. (2009) who reported genetic correlation between EV and WBSF of 0.24 and 0.35. The correlations are opposite in sign because a higher EV results in a lower FT. Kadel et al. (2006) also estimated a phenotypic correlation of -0.02 and -0.04 between WBSF and temperament measured as EV taken post-weaning and WBSF and EV taken at the start of

finishing, respectively. Ribeiro et al. (2007) concluded that WBSF values were 10.7 to 19.3% lower in calmer steers.

Café et al. (2010) looked for the effects of SNP markers for tenderness on temperament in a Brahman cattle herd. *Bos indicus* breeds have greater calpastatin activity than do *Bos taurus* breeds, which may explain why they also produce tougher steaks. Four tenderness markers were used in the study; calpastatin (*CAST*), calpain 3 (*CAPN3*), and two markers in the  $\mu$ -calpain region (*CAPN1-4851* and *CAPN1-316*) to determine marker effects on temperament. *CAPN1-4751* was the only marker to be associated with both tenderness and temperament. With two favorable alleles for WBSF at *CAPN1-4751*, animals had a greater EV than those with zero or one favorable allele. *CAPN3* showed a tendency ( $p = 0.08$ ) for cattle with one favorable WBSF allele to have a lower EV. For this study, selection to improve tenderness in Brahman cattle using favorable *CAPN1-4751* and *CAPN3* alleles would have favorable effects on temperament.

## Research Objectives

Chapter 2 explores the phenotypic and genetic correlations between temperament and tenderness in *Bos taurus* cattle, specifically in purebred Angus, Simmental, and Angus  $\times$  Simmental crossbred animals. The first objective was to investigate and compare statistical methods to analyze the dataset, which included multiple records for both temperament and tenderness. Secondly, the statistical models were used to estimate genetic parameters, including heritability and genetic and phenotypic correlations, among the two traits. Results from these studies could provide tools suitable for selection of animals that influence producer profit and herd performance.

Chapter 3 describes a genome-wide association study (GWAS) performed using the Illumina BovineSNP50 BeadArray (San Diego, CA, USA; Matikumalli et al., 2009). The objective of the study was to conduct a GWAS to detect single-nucleotide polymorphism (SNP) markers associated with temperament or tenderness in Simmental  $\times$  Angus crossed cattle. Strong associations between SNP markers in linkage disequilibrium with particular quantitative trait loci may provide some evidence of a candidate gene harbored in the region.

The overall goal of the study was to develop the most accurate estimates of genetic merit for each animal. These estimates will enhance a producer's ability to improve herd performance and net profit. Understanding the nature of correlations between traits will present the possibility of influencing one trait with an early selection process on the other trait. For example, if there is a positive genetic correlation between the traits, selection on temperament could be implemented at weaning, which then would

influence tenderness, which is measured at the end of the animal's life. Increasing the accuracy of genetic predictions, by utilizing marker-assisted selection through molecular breeding values, may allow for faster genetic improvement in a herd.

**Table 1.1.** Chute and Pen score rubrics

	Chute Scoring Rubric	Pen Scoring Rubric
1	<p>Docile:</p> <p>Mild disposition, gentle and easily handled, stands and moves slowly, undisturbed, settled, somewhat dull.</p> <p>Exits chute calmly.</p>	<p>Docile:</p> <p>Walks slowly, can approach closely, not excited by humans or facilities.</p>
2	<p>Restless:</p> <p>Quieter than average, may be stubborn, may try to back out of chute, some flicking of tail.</p> <p>Exits chute promptly.</p>	<p>Slightly Aggressive:</p> <p>Runs along fences, will stand in corner if humans stay away, may pace fence.</p>
3	<p>Nervous:</p> <p>Typical temperament is manageable, nervous and impatient, moderate amount of struggling, movement and tail flicking.</p> <p>Exits chute briskly.</p>	<p>Moderately Aggressive:</p> <p>Runs along fences, head up and will run if humans move closer, stops before hitting gates and fences, avoids humans.</p>
4	<p>Flighty:</p> <p>Jumpy and out of control, quivers and struggles violently, may bellow or froth at the mouth, continuous tail flicking, defecates and urinates.</p> <p>Exits chute wildly.</p>	<p>Aggressive:</p> <p>Runs, stays in back of group, head high and very aware of humans, may run into fences and gates even with some distance, will likely run into fences if alone in pen.</p>
5	<p>Aggressive:</p> <p>May be similar to score 4, but with added aggressive behavior, fearfulness, extreme agitation, and continuous movement which may include jumping and bellowing while in chute.</p> <p>Exits chute frantically and may exhibit attack behavior when handled.</p>	<p>Very Aggressive:</p> <p>Excited, runs into fences, runs over humans and anything else in path, "crazy."</p>
6	<p>Very Aggressive:</p> <p>Extremely aggressive temperament. Thrashes about or attacks wildly when confined. Pronounced attack behavior.</p>	

# **CHAPTER 2**

## **QUANTITATIVE ANALYSIS OF PHENOTYPIC AND GENETIC RELATIONSHIPS BETWEEN TEMPERAMENT AND TENDERNESS TRAITS IN BEEF CATTLE**

### **Summary**

Tenderness is a primary meat palatability attribute affecting the consumer satisfaction of beef. Beef cattle temperament has been associated with a variety of performance measures. Australian researchers found a strong negative association (-0.54) between Warner-Bratzler shear force (WBSF) and flight times for tropically adapted *Bos indicus* influenced breeds. Performance data and pedigree records were provided by the American Simmental Association (ASA) to elucidate the relationship between temperament and tenderness in *Bos taurus* breeds. Data included WBSF records from ASA's carcass merit program and a subset collected at the University of Illinois. Exit velocities were recorded when cattle went on trial (EV1) and 42 days later (EV2). Single animal and single sire contemporary groups (CG) were removed from the data set leaving 2,819 WBSF, 917 EV1, and 976 EV2 phenotypes in 176 CG for evaluation. A pedigree was formed with 13,418 animals including 2,488 sires. Phenotypic means  $\pm$  standard

deviation were  $3.74 \pm 1.08$ -kg for WBSF,  $1.74 \pm 0.76$ -m/s for EV1 and  $1.65 \pm 0.79$ -m/s for EV2. A tri-variate animal model with CG, sire breed composition, and dam breed composition as fixed effects and animal as random effect was fit to estimate variance components. Phenotypic correlations  $\pm$  standard error estimated between WBSF with EV1 and EV2 were  $-0.05 \pm 0.05$  and  $-0.03 \pm 0.04$ , respectively, and between EV1 and EV2 was  $0.59 \pm 0.02$ . Heritabilities  $\pm$  standard error for WBSF, EV1 and EV2 were  $0.19 \pm 0.06$ ,  $0.30 \pm 0.11$  and  $0.25 \pm 0.10$ , respectively. Genetic correlations estimated between WBSF with EV1 and EV2 were  $0.02 \pm 0.38$  and  $-0.30 \pm 0.36$  respectively. Given the high genetic correlation between EV1 and EV2 of  $0.99 \pm 0.07$  a repeated records analysis was performed for EV with an uncorrelated random effect for animal using the same fixed effects which provided a better model fit. Heritability estimates were  $0.19 \pm 0.06$  and  $0.39 \pm 0.08$  for WBSF and EV with a genetic correlation of  $-0.10 \pm 0.20$ . The near zero genetic correlation and moderate heritability estimates suggest that producers can select to improve temperament and/or WBSF without a substantial correlated response in the second trait.



## Introduction

Beef producers constantly search for new selection strategies which will affect herd performance and improve profitability. Temperament is a trait of recent interest to producers. Not only are producers interested in improving the behavior of their animals, they are concerned with the impact that selection of temperament will have on other correlated traits. Australian researchers have found an association between temperament and steak tenderness. A 0.24 to 0.35 correlation was recorded between temperament (exit velocity; EV) and steak tenderness (Warner-Bratzler shear force; WBSF) in tropically adapted *Bos indicus* influenced cattle breeds (Behrends et al., 2009). Temperament, measured as EV, is best measured before cattle acclimate to the production system, and therefore can be recorded at weaning (Behrends et al., 2009). Warner-Bratzler shear force is widely used to measure the tenderness of meat, which has been shown to be one of the highest beef quality concerns influencing consumer acceptability of beef (Crouse et al., 1989; Lusk et al., 2001). If this magnitude and direction of correlation between temperament and tenderness exists in *Bos taurus* cattle, it will be possible for producers to make early selection decisions that favorably influence their herd performance. Industry wide improvements in WBSF may increase the demand for beef and prices received by producers (Weaber and Lusk, 2010). Exit velocity and WBSF have been analyzed using repeated measures mixed models as well as multi-variate linear mixed models (Burrow et al., 2001; Curley et al., 2006; Kadel et al., 2006; Burdick et al., 2009; Café et al., 2010; Weaber and Creason, 2010). The objectives of this paper were to: (1) investigate and compare statistical methods to analyze the dataset, which included

multiple records for both traits; and (2) estimate genetic parameters, including heritabilities, genetic and phenotypic correlations for tenderness and temperament traits.

## Materials and Methods

### Record Collection

Data for this study were provided by the American Simmental Association (ASA), and included performance records collected from 2001 through 2008 (Pollak et al., 2001). These included records from ASA's Carcass Merit Program ( $n = 3,776$ ). The animals were purebred Angus, purebred Simmental, or Angus  $\times$  Simmental crossbreds. Performance records included a temperament measure and a steak tenderness measure. Cattle temperament is most usefully measured objectively as an exit velocity (EV) which has been shown to be better than any of the other subjective measures (Curley et al., 2006). Exit velocity is the rate (m/s) at which an animal exits the working chute and covers a distance of 1.7-m (Burrow et al., 1988; Curley et al., 2006). Elapsed time was recorded in thousandths of a second by a simple electronic system (Polaris Timing System, FarmTek, Wylie, TX). Two light beams are focused on infra-red sensors spaced at the chute (head bail) and 1.7-m away from the head bail. The sensors trigger an on/off mechanism, so that as the animal breaks the light beam, the timing apparatus stops and a connected computer records the time (Burrow et al., 1988; Curley et al., 2006; Beckman et al., 2007). The elapsed time required for the animal to pass between the two sets of infra-red sensors is recorded, and is termed the animal's flight time (FT). Exit velocity is computed as the distance traversed divided by FT and is reported as m/s (Beckman et al., 2007). In this study, EV was measured at most of two times, on the first day of a feeding trial (EV1) and 42 days (EV2) later at the midpoint of the trial. Warner-Bratzler shear force (WBSF) is the most widely used measure of beef tenderness. The protocol used for

all WBSF measurements followed the protocol outlined by Dikeman et al. (2005), which closely follows the American Meat Science Association (AMSA) recommendations as well as the proposed shear force procedures for meat tenderness measurements (Wheeler et al., 2005). The *longissimus dorsi* (LD) muscle was collected at the slaughter plant, and on return to the research institution, 2.54-cm steaks were cut and immediately vacuum-packaged. The steaks were aged at 2°C for 14 days and then thawed for 24 hours. The steaks were then cooked on a convection conveyor oven (XLT Oven Model 1832-EL, BOFI, Inc., Wichita, KS) to an internal temperature of 71°C (medium degree of doneness). After the steaks were taken off the conveyor oven, internal temperatures were measured with a hand-held thermometer using a wire thermocouple (HH-21, Omega Engineering, Stamford, CT USA), and after the post-cooking temperature rise was complete, this temperature was recorded in order to adjust the WBSF measurement. The steaks were next chilled at 2°C for 24 hours. An average of eight 1.27-cm steak cores were removed parallel to the muscle fiber orientation of each steak using a hand-held coring device. Each core was then sheared using a United-Smart 1 Test System SSTM – 500 (United Calibration Corp., Huntington Beach, CA) with a head speed of 250-mm/min, and shear force was recorded for each core.

## Statistical Models

Over the years during which data were collected there was variation in the amount of information collected on each animal, creating missing observations. Observations taken on animals during the earlier years included only an EV1 measurement, whereas later born animals had both EV1 and EV2 measurements recorded. Animals had WBSF observations collected from 1 to 12 steak cores, with an average of 7.52 cores per steak. Average peak shear force (APSF) was calculated as the average of all cores taken on an individual animal. Because each animal had the possibility for multiple measurements in both performance traits, 4 statistical models were evaluated to determine the best models of this data. Table 2.1 provides the counts of animals cross classified with various measurements available in the dataset. Contemporary groups (CG) were assigned by providing a unique identifier for animals with common year of birth and herd of origin. After single animal and single sire CGs were removed, the dataset included 3,042 total animals with 13,418 animals represented in a 29 generation pedigree. The pedigree file included 2,488 sires, 1,115 paternal grand-sires, 1,671 paternal grand-dams, 6,952 dams, 1,580 maternal grand-sires, and 3,663 maternal grand-dams. Each of the 176 CGs consisted of an average of 17.28 animals, but ranged from 2 to 126 animals. ASREML software version 3.0 (VSN International Ltd., Hemel Hempstead, UK) was utilized to estimate phenotypic and genetic variances as well as heritability.

*Analysis of APSF with comparison of EV fit as two traits or repeated records*

Exit velocity was measured at most two times; therefore EV could be analyzed as two independent traits or as a repeated record for a single trait. These models were both analyzed to determine the best fit model for EV records. The first model (Model 1) was a tri-variate animal model which included APSF, EV1, and EV2. The dataset consisted of 3,042 total animals in 176 different CGs with 2,819 APSF, 917 EV1, and 976 EV2 observations.

Phenotypic and genetic variance components for APSF, EV1, and EV2 in Model 1 were estimated using the following model (Mrode, 2005):

$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \end{bmatrix} = \begin{bmatrix} X_1 & 0 & 0 \\ 0 & X_2 & 0 \\ 0 & 0 & X_3 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \\ b_3 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 & 0 \\ 0 & Z_2 & 0 \\ 0 & 0 & Z_3 \end{bmatrix} \begin{bmatrix} u_1 \\ u_2 \\ u_3 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \\ e_3 \end{bmatrix}$$

where  $\mathbf{y}$  is a vector of phenotypes (APSF, EV1, and EV2),  $\mathbf{b}$  is a vector of fixed effects (CG, Sire Breed, and Dam Breed),  $\mathbf{u}$  is a vector of random animal effects,  $\mathbf{X}$  and  $\mathbf{Z}$  are incidence matrices that relate each trait to fixed effects and random effects, respectively, and  $\mathbf{e}$  is a vector of residual effects.

The assumed Model 1 variance was:

$$\text{var} \begin{bmatrix} u_1 \\ u_2 \\ u_3 \\ e_1 \\ e_2 \\ e_3 \end{bmatrix} = \begin{bmatrix} g_{11}A & g_{12}A & g_{13}A & 0 & 0 & 0 \\ g_{12}A & g_{22}A & g_{23}A & 0 & 0 & 0 \\ g_{13}A & g_{23}A & g_{33}A & 0 & 0 & 0 \\ 0 & 0 & 0 & Ir_{11}I & r_{12} & Ir_{13} \\ 0 & 0 & 0 & r_{12} & Ir_{22} & Ir_{23} \\ 0 & 0 & 0 & Ir_{13} & Ir_{23} & Ir_{33} \end{bmatrix}$$

where  $\text{Var}(\mathbf{u}) = \mathbf{G}$  is the additive genetic variance and covariance matrix for animal effects with each element defined as  $g_{ij}$  ( $g_{11}$  is the additive genetic variance for direct effects in trait 1,  $g_{12}$  is the additive genetic covariance for direct effects between traits 1 and 2),  $A$  is the numerator relationship matrix among animals,  $I$  is the identity matrix, and  $\text{Var}(\mathbf{e}) = \mathbf{R} = \{r_{ij}\}$  is the variance and covariance matrix for residual effects.

The mixed model equation (MME) can be written as:

$$\begin{bmatrix} X'R^{-1}X & X'R^{-1}Z' \\ Z'R^{-1}X & Z'R^{-1}Z + A^{-1} \times G^{-1} \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{u} \end{bmatrix} = \begin{bmatrix} X'R^{-1}y \\ Z'R^{-1}y \end{bmatrix}$$

where:

$$X = \begin{bmatrix} X_1 & 0 & 0 \\ 0 & X_2 & 0 \\ 0 & 0 & X_3 \end{bmatrix}, Z = \begin{bmatrix} Z_1 & 0 & 0 \\ 0 & Z_2 & 0 \\ 0 & 0 & Z_3 \end{bmatrix}, \hat{b} = \begin{bmatrix} \hat{b}_1 \\ \hat{b}_2 \\ \hat{b}_3 \end{bmatrix}, \hat{u} = \begin{bmatrix} \hat{u}_1 \\ \hat{u}_2 \\ \hat{u}_3 \end{bmatrix}, \text{ and } y = \begin{bmatrix} y_1 \\ y_2 \\ y_3 \end{bmatrix}$$

and  $y$  is an ordered vector of phenotypes for APSF, EV1, and EV2,  $\hat{b}$  is a vector of fixed effect solutions,  $\hat{u}$  is a vector of random animal effect solutions, and  $X$  and  $Z$  are block diagonal matrices of incidence matrices relating animals to fixed effects and random effects, respectively.

Model 2 was a repeated records animal model which included APSF and EV with EV1 and EV2 acting as repeated observations on EV. Model 2 had the same 3,042

animals as Model 1 in 176 different CGs with 2,819 APSF, and 998 animals with at least one EV observation (from the 917 EV1 and 976 EV2) which was modeled as a repeated record trait.

Phenotypic and genetic variance components for APSF and EV in Model 2 were estimated using the following model (Mrode, 2005):

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} u_1 \\ u_2 \end{bmatrix} + \begin{bmatrix} 0 & 0 \\ 0 & S_2 \end{bmatrix} \begin{bmatrix} 0 \\ pe_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

where  $\mathbf{y}$  is the vector of observations for trait 1 (APSF) and trait 2 (EV),  $\mathbf{b}$  is a vector of fixed effects (CG, Sire Breed, and Dam Breed),  $\mathbf{u}$  is a vector of random animal effects,  $\mathbf{pe}$  is a vector of random permanent environmental effects (not estimable for APSF),  $\mathbf{X}$ ,  $\mathbf{Z}$ , and  $\mathbf{S}$  are incidence matrices that relate each trait to fixed effects, random effects, and permanent environmental effects, respectively, and  $\mathbf{e}$  is a vector of residual effects.

The assumed model variance was:

$$\text{var} \begin{bmatrix} u_1 \\ u_2 \\ pe_2 \\ e_1 \\ e_2 \end{bmatrix} = \begin{bmatrix} g_{11}Ag_{12}A & 0 & 0 & 0 \\ g_{12}Ag_{22}A & 0 & 0 & 0 \\ 0 & 0 & Iq_{22} & 0 \\ 0 & 0 & 0 & Ir_{11} & Ir_{12} \\ 0 & 0 & 0 & Ir_{21} & Ir_{22} \end{bmatrix}$$

where  $\mathbf{G} = \{g_{ij}\}$  is the additive genetic variance and covariance matrix for animal effects,  $\mathbf{A}$  is the numerator relationship matrix among animals,  $\mathbf{Q}$  (containing the element  $q_{22}$ ) is the variance and covariance matrix for permanent environmental effects,  $\mathbf{pe}$ ,  $\mathbf{I}$  is the identity matrix,  $\mathbf{R} = \{r_{ij}\}$  is the variance and covariance matrix for residual effects, and  $\mathbf{u}$  and  $\mathbf{e}$  are as previously described.



The mixed model equations can be written as:

$$\begin{bmatrix} X'R^{-1}X & X'R^{-1}Z & X'R^{-1}S \\ Z'R^{-1}X & Z'R^{-1}Z + k_1 & Z'R^{-1}S \\ S'R^{-1}X & S'R^{-1}Z & S'R^{-1}S + I * Q^{-1} \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{u} \\ \widehat{pe} \end{bmatrix} = \begin{bmatrix} X'R^{-1}y \\ Z'R^{-1}y \\ S'R^{-1}y \end{bmatrix}$$

where:

$$y = \begin{bmatrix} y_1 \\ y_2 \end{bmatrix}, \hat{b} = \begin{bmatrix} \hat{b}_1 \\ \hat{b}_2 \end{bmatrix}, \hat{u} = \begin{bmatrix} \hat{u}_1 \\ \hat{u}_2 \end{bmatrix}, \widehat{pe} = \begin{bmatrix} \widehat{pe}_1 \\ \widehat{pe}_2 \end{bmatrix},$$

$$X = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix}, Z = \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix}, S = \begin{bmatrix} 0 & 0 \\ 0 & S_2 \end{bmatrix}$$

$$k_1 = G_1 * A^{-1}$$

with:

$$G_1 = \begin{bmatrix} g^{11} & g^{12} \\ g^{12} & g^{22} \end{bmatrix} \text{ the inverse of } \begin{bmatrix} g_{11} & g_{12} \\ g_{12} & g_{22} \end{bmatrix}.$$

here  $y$  is an ordered vector of phenotypes for APSF and EV,  $\hat{b}$  is a vector of fixed effect solutions,  $\hat{u}$  is a vector of random animal effect solutions, and  $\widehat{pe}$  is a vector of permanent environmental random effect solutions.  $X$  and  $Z$  are block diagonal matrices of incidence matrices relating animals to fixed effects and random effects, respectively.  $S$  is a block diagonal matrix of incidence matrices relating animals to permanent environmental random effects describing the covariance among repeated phenotypes within animal, and  $G$  is the additive genetic variance and covariance matrix.  $A^{-1}$  is the inverse of the numerator relationship matrix.  $R^{-1}$  is the inverse of the residual variance and covariance matrix.

The Bayesian Information Criteria (BIC) was used to evaluate which model provided the best fit to the data (Gilmour et al., 2006). BIC was computed for each model and the model with the smallest BIC was chosen as the preferred model.

BIC was computed as:

$$BIC = -2\ell_{Ri} + \tau_i \log v$$

where  $\ell_{Ri}$  is the model log-likelihood,  $\tau_i$  is the number of variance parameters in the model, and  $v = n - p$  the residual degrees of freedom.

*Analysis of WBSF as mean of records or as repeated records with EV as repeated*

Warner-Bratzler shear force could be analyzed as one trait (APSF) or as a repeated record (WBSF) based on the shear values of individual cores. The dataset was reduced to only include animals with multiple core values, and EV was analyzed as a repeated record trait. The reduced dataset included observations on 1,871 animals in 128 different CGs. The following models utilized this reduced dataset, and therefore cannot be directly compared by likelihood to the results from Models 1 and 2. The full model (Model 3) included 1,871 animals of which 1,204 animals had multiple WBSF values (8,960 total WBSF observations recorded), and 998 animals with at least one EV observation which was modeled as a repeated record trait.

Phenotypic and genetic variance components for WBSF and EV were estimated using the following model (Mrode, 2005):

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} u_1 \\ u_2 \end{bmatrix} + \begin{bmatrix} S_1 & 0 \\ 0 & S_2 \end{bmatrix} \begin{bmatrix} pe_1 \\ pe_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

where  $\mathbf{y}$  is the vector of observations for trait 1 (WBSF) and trait 2 (EV),  $\mathbf{b}$  is a vector of fixed effects (CG, Sire Breed, and Dam Breed),  $\mathbf{u}$  is a vector of random animal effects,  $\mathbf{pe}$  is a vector of random permanent environmental effects,  $\mathbf{X}$ ,  $\mathbf{Z}$ , and  $\mathbf{S}$  are incidence matrices that relate each trait to fixed effects, random effects, and permanent environmental effects, respectively, and  $\mathbf{e}$  is a vector of residual effects.

The assumed model variance was:

$$var \begin{bmatrix} u_1 \\ u_2 \\ pe_1 \\ pe_2 \\ e_1 \\ e_2 \end{bmatrix} = \begin{bmatrix} g_{11}Ag_{12}A & 0 & 0 & 0 & 0 & 0 \\ g_{12}Ag_{22}A & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & Iq_{11}Iq_{12} & 0 & 0 & 0 \\ 0 & 0 & Iq_{21}Iq_{22} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & I r_{11}I r_{12} & 0 \\ 0 & 0 & 0 & 0 & I r_{12}I r_{22} & 0 \end{bmatrix}$$

where  $\mathbf{G} = \{g_{ij}\}$  is the additive genetic variance and covariance matrix for animal effects,

$\mathbf{A}$  is the numerator relationship matrix among animals,  $\mathbf{Q} = \{q_{ij}\}$  is the variance and covariance matrix for permanent environmental effects( $\mathbf{pe}$ ),  $\mathbf{I}$  is the identity matrix,

$\mathbf{R} = \{r_{ij}\}$  is the variance and covariance matrix for residual effects, and  $\mathbf{u}$  and  $\mathbf{e}$  are as previously described.

The mixed model equation can be written as:

$$\begin{bmatrix} X'R^{-1}X & X'R^{-1}Z & X'R^{-1}S \\ Z'R^{-1}X & Z'R^{-1}Z + k_1 & Z'R^{-1}S \\ S'R^{-1}X & S'R^{-1}Z & S'R^{-1}S + I * Q^{-1} \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{u} \\ \widehat{pe} \end{bmatrix} = \begin{bmatrix} X'R^{-1}y \\ Z'R^{-1}y \\ S'R^{-1}y \end{bmatrix}$$

where:

$$y = \begin{bmatrix} y_1 \\ y_2 \end{bmatrix}, \hat{b} = \begin{bmatrix} \hat{b}_1 \\ \hat{b}_2 \end{bmatrix}, \hat{u} = \begin{bmatrix} \hat{u}_1 \\ \hat{u}_2 \end{bmatrix}, \widehat{pe} = \begin{bmatrix} \widehat{pe}_1 \\ \widehat{pe}_2 \end{bmatrix},$$

$$X = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix}, Z = \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix}, S = \begin{bmatrix} S_1 & 0 \\ 0 & S_2 \end{bmatrix}$$

$$k_1 = G_1 * A^{-1}$$

with:

$$G_1 = \begin{bmatrix} g^{11} & g^{12} \\ g^{12} & g^{22} \end{bmatrix} \text{ the inverse of } \begin{bmatrix} g_{11} & g_{12} \\ g_{12} & g_{22} \end{bmatrix}.$$

here  $\mathbf{y}$  is an ordered vector of phenotypes for WBSF and EV,  $\hat{\mathbf{b}}$  is a vector of fixed effect solutions,  $\hat{\mathbf{u}}$  is a vector of random animal effect solutions, and  $\hat{\mathbf{pe}}$  is a vector of permanent environmental random effect solutions.  $\mathbf{X}$  and  $\mathbf{Z}$  are block diagonal matrices of incidence matrices relating animals to fixed effects and random effects, respectively.  $\mathbf{S}$  is a block diagonal matrix of incidence matrices relating animals to permanent environmental random effects describing the covariance among repeated phenotypes within animal, and  $\mathbf{G}$  is the additive genetic variance and covariance matrix.  $\mathbf{A}^{-1}$  is the inverse of the numerator relationship matrix.  $\mathbf{R}^{-1}$  is the inverse of the residual variance and covariance matrix.

The reduced model (Model 4) included the same 1,871 animals as Model 3. APSF was calculated from the 8,690 WBSF values on 1,204 animals, and the same 998 animals with at least one EV observation from Model 3 were analyzed as a repeated record trait. This model followed the same parameterization as previously described for Model 2, but was applied to a reduced dataset. The variance components for APSF and EV were estimated using the model previously described in Model 2. A comparison of BIC values was used to determine whether the full (Model 3) or the reduced model (Model 4) provided the best data fit (Gilmour et al., 2006).

## Results and Discussion

Table 2.2 shows the count of observations for each trait used in each statistical model. Wheeler et al. (1997) concluded that steak tenderness was considered acceptable to consumers if it was less than 4.30-kg and unacceptable if greater than this amount. The APSF in this dataset ranged from an acceptable (1.43-kg) to an unacceptable (6.61-kg) and was nearly normally distributed as demonstrated in Figure 2.1. Average peak shear force measurements in other studies were 4.41-kg and 4.49-kg in purebred Angus and Simmental herds, respectively (Burrow et al., 2001). A low percentage (6%) of the steaks in this study would have been considered unsatisfactory by consumers. In retail, 2 to 11% of the steaks are classified as unsatisfactory, because the APSF exceeds 4.30-kg (Savell et al., 2006; Wheeler et al., 1997).

Behrends et al. (2009) recorded EV1 in the range from 1.19 to 5.85-m/s and EV2 to range from 1.01 to 5.24-m/s in *Bos indicus* cattle. Exit velocity 1 ranged from 0.07 to 4.48-m/s, and EV2 ranged from 0.09 to 4.63-m/s. The EV data were slightly bimodal, shown in Figure 2.2, but no transformation to the data was performed, therefore a normal distribution was assumed. Phenotypic means and standard deviations for EV1, EV2, and APSF were  $1.74 \pm 0.76$ -m/s,  $1.65 \pm 0.79$ -m/s, and  $3.74 \pm 1.08$ -kg, respectively. In *Bos indicus* crossed breeds, the standard deviation was found to be higher for the second measurement, but this was not seen in this dataset. *Bos indicus* cattle are known to have poorer temperaments than *Bos taurus* cattle and therefore may have less ability to habituate to handling. The dataset used in this study contains only *Bos taurus* animals which had similar EV and standard deviation observations at both observation times.

Reverter et al. (2003) found phenotypic correlations between FT and APSF were near zero and supported by this data (Figure 2.3).

## Exit Velocity Models

The EV models were examined to determine the best fit model for the multiple EV observations. Results from Model 1 (tri-variate animal model) are shown in Table 2.3 and are in agreement with previous genetic correlation estimates between EV1 and EV2 which range from 0.60 to 0.78 in *Bos indicus* crossbreds (Burrow and Dillion, 1997). The strong correlations between EV measurements in Model 2 were of interest. Model 2 estimated near zero phenotypic and genetic correlation between APSF and EV, as shown in Table 2.4. Heritability estimates were  $0.19 \pm 0.06$  and  $0.39 \pm 0.09$  for APSF and EV, respectively. The comparison of Bayesian Information Criteria (BIC) values was used to conclude that Model 2, the two trait model with EV as a repeated record is preferred over Model 1 (Table 2.7). Burrow and Dillion (1997) also concluded that FT was best analyzed as a repeated record. Behrends et al. (2009) found genetic correlations of 0.24 and 0.35 between EV and APSF using two EV measurements. The genetic correlation between FT and APSF was -0.42 when FT was taken post-weaning and -0.32 when FT was taken at the start of finishing. Reverter et al. (2003) and Kadel et al. (2006) reported a near zero phenotypic correlation between APSF and FT. The lower genetic correlations estimated in these studies suggest that *Bos taurus* breeds do not behave the same as do *Bos indicus* crossed breeds. Exit velocity has a heritability of 0.40 in *Bos indicus* breeds, 0.36 in the Canadian Beef Cattle Reference herd, and 0.35 among *Bos taurus* breeds (Burrow and Corbet, 2000; Beckman et al., 2007). When EV is taken at weaning, Burrow et al. (1988) found a heritability of 0.54 in *Bos indicus* breeds. Other studies have found similar heritability estimates for temperament using EV; 0.49 in *Bos taurus* breed crosses



and 0.31 in *Bos taurus* and *Bos indicus* breed crosses (Johnston et al., 2003a; Reverter et al., 2003; Kadel et al., 2006; Nkrumah et al., 2007). Animals with higher *Bos indicus* content tend to have a higher heritability for temperament (Burrow and Corbet, 2000) than *Bos taurus* breeds.

### Warner-Bratzler Shear Force Models

The previous two statistical models analyzed EV as two correlated traits and as a single trait with repeated records. The repeated record model provided a better fit. This result motivated to consider WBSF in a similar fashion. Warner-Bratzler shear force is recorded as multiple core values taken from the same steak. The following models analyze WBSF as a single observation of average peak shear force (APSF), and as a repeated record (WBSF) to determine the best fit for WBSF observations. The dataset was reduced to include only animals that have multiple WBSF core value observations, as previously explained. Four to 12 steak cores, an average of 7.52 cores, were taken from the 1,871 animals. Model 3 (WBSF and EV as repeated records) estimated near zero phenotypic and genetic correlations between WBSF and EV. Heritability for WBSF was near zero, but EV was moderately heritable, as shown in Table 2.5. Model 4 (APSF, EV as repeated record) estimates were similar to results of Model 3, as shown in Table 2.6. The phenotypic and genetic correlations are near zero, as estimated in the previous models, Model 1 and Model 2 where EV was analyzed as a single record or repeated record, respectively. Heritability for WBSF and APSF was near zero in both models, and because the same EV observations were used in both Models 3 and 4, heritability estimates were equivalent. The comparison of BIC values was used to conclude that Model 4 is preferred over Model 3 (Table 2.7). The APSF or WBSF heritability estimates for Models 3 and 4 were near zero. Given the results from Models 1 and 2 which included more observations, the lower heritability is most likely due to data sampling issues. There is a trend for Simmental cattle to have lower heritability for WBSF. Minick et al. (2004)

found Angus-sired steers to have a heritability of  $0.33 \pm 0.25$ , while records on Simmental-sired steers yielded a lower heritability ( $0.16 \pm 0.14$ ) estimate for APSF. There is a close agreement between heritability estimates ( $0.31 \pm 0.03$ ) found in temperate and tropically adapted breeds of cattle (Burrow et al., 1988; Burrow and Corbet, 2000; Johnston et al., 2003a). Another paper estimated the heritability of APSF in Simmental-sired cattle to be 0.08 (McClure et al., in press). Repeatability of WBSF was estimated from Model 3 to be 0.85 and 0.84 for EV. The repeatability of FT in a *Bos indicus* crossbred study was estimated to be  $0.88 \pm 0.01$  (Burrow and Dillion, 1997). These results suggest that only small increases in accuracy could be expected through the use of repeated measures of EV.

While there is a strong genetic relationship between APSF and EV in *Bos indicus* crossbred steers (Behrends et al., 2009; Burrows et al., 2001; Johnston 2003b), this study found almost no relationship in *Bos taurus*. The near zero phenotypic and genetic correlations estimated between beef tenderness and temperament in *Bos taurus* cattle suggest that producers can select to improve either trait without observing a correlated response in the other. The lack of any genetic relationship also means that producers should not use EV as an indicator trait for WBSF. Selection for lower EV is not expected to result in improvement in WBSF.

**Table 2.1** Total animal counts cross classified with phenotypic observations.

Warner-Bratzler Shear Force	Exit Velocity 1 <sup>1</sup>	Exit Velocity 2 <sup>1</sup>	Total Animal Count
✓			2,044
✓	✓		15
✓		✓	75
✓	✓	✓	685
	✓		7
		✓	6
	✓	✓	210

<sup>1</sup>Exit velocity 1 measurement was taken on the first day of a feeding trial, Exit velocity 2 measurement was taken 42 days after exit velocity 1.

**Table 2.2.** Statistical models used to analyze average peak shear force (APSF), Warner-Bratzler shear force (WBSF), exit velocity 1 (EV1)<sup>1</sup>, and exit velocity 2 (EV2)<sup>2</sup> and the corresponding number of observations for each trait.

Statistical Model <sup>3</sup>	Animals with Records in Performance File	Contemporary Groups	WBSF Core Values	APSF	Animals with WBSF Cores of APSF	EV1	EV2	Animals with One or More EV
Model 1	3,042	176		2,819		917	976	998
Model 2	3,042	176		2,819		917	976	998
Model 3	1,871	128	8,690		1,204	917	976	998
Model 4	1,871	128		1,204	1,204	917	976	998

<sup>1</sup> Exit velocity 1 measurements were taken the first day of a feeding trial.

<sup>2</sup> Exit velocity 2 measurements were taken 42 days after exit velocity 1 (EV 1).

<sup>3</sup> Model 1 (n = 3,042) was a tri-variate animal model including APSF, EV1, and EV2.  
Model 2 (n = 3,042) analyzed APSF and exit velocity as a repeated record of EV1 and EV2.  
Model 3 (n = 1,871) analyzed WBSF and EV as repeated record.  
Model 4 (n = 1,871) analyzed APSF and EV as a repeated record.

**Table 2.3.** Model 1<sup>1</sup> estimates of heritability, phenotypic and genetic correlations<sup>2</sup>  $\pm$  standard error between average peak shear force (APSF), exit velocity 1 (EV1), and exit velocity 2 (EV2)<sup>3</sup>.

	APSF	EV1	EV2
APSF	0.19 $\pm$ 0.06	-0.05 $\pm$ 0.05	-0.03 $\pm$ 0.04
EV1	0.02 $\pm$ 0.38	0.30 $\pm$ 0.11	0.59 $\pm$ 0.02
EV2	-0.30 $\pm$ 0.36	0.99 $\pm$ 0.07	0.25 $\pm$ 0.10

<sup>1</sup> Model 1 (n = 3,042) was a tri-variate animal model which analyzed APSF, EV1, and EV2 as single trait variables.

<sup>2</sup> Phenotypic correlations are shown in the upper right portion of the table, genotypic correlations are in the bottom left portion of the table, and heritability estimates are shown on the diagonal.

<sup>3</sup> A log likelihood value of -6,662.71 was estimated at convergence.

**Table 2.4.** Model 2<sup>1</sup> estimates of heritability, phenotypic and genetic correlations<sup>2</sup>  $\pm$  standard error between average peak shear force (APSF) and exit velocity (EV)<sup>3</sup>.

	APSF	EV
APSF	0.19 $\pm$ 0.06	-0.08 $\pm$ 0.06
EV	-0.10 $\pm$ 0.20	0.39 $\pm$ 0.09

<sup>1</sup> Model 2 (n = 3,029) was a bivariate animal model which analyzed APSF as a single trait and EV as a repeated record trait.

<sup>2</sup> Phenotypic correlations are shown in the upper right corner of the table, genotypic correlations are in the bottom left corner of the table, and heritability estimates are shown on the diagonal.

<sup>3</sup> A log likelihood value of -6,575.00 was estimated at convergence.

**Table 2.5.** Model 3<sup>1</sup> estimates of heritability, phenotypic and genetic correlations  $\pm$  standard error between Warner-Bratzler shear force (WBSF) and exit velocity (EV).

	WBSF	EV
WBSF	$0.06 \pm 0.05$	$-0.09 \pm 0.06$
EV	$-0.63 \pm 0.47$	$0.38 \pm 0.09$

<sup>1</sup> Model 3 (n = 1,871) used a reduced dataset and analyzed WBSF as a repeated record and EV as a repeated record.

<sup>2</sup> Phenotypic correlations are shown in the upper right corner of the table, genotypic correlations are in the bottom left corner of the table, and heritability estimates are shown on the diagonal.

<sup>3</sup> A log likelihood value of -3,703.94 was estimated at convergence.



**Table 2.6.** Model 4<sup>1</sup> estimates of heritability, phenotypic and genetic correlations<sup>2</sup>  $\pm$  standard error between average peak shear force (APSF) and exit velocity (EV).

	APSF	EV
APSF	0.06 $\pm$ 0.06	-0.09 $\pm$ 0.06
EV	-0.62 $\pm$ 0.47	0.38 $\pm$ 0.09

<sup>1</sup> Model 4 (n = 1,871) used a reduced dataset and was a two trait animal model which analyzed APSF as a single trait and EV as a repeated record.

<sup>2</sup> Phenotypic correlations are shown in the upper right corner of the table, genotypic correlations are in the bottom left corner of the table, and heritability estimates are shown on the diagonal.

<sup>3</sup> A log likelihood value of -2,754.44 was estimated at convergence.

**Table 2.7.** Log-likelihood at convergence, number of variance parameters estimated and residual degrees of freedom, and Bayesian Information Criteria (BIC) for models of tenderness and temperament evaluated.

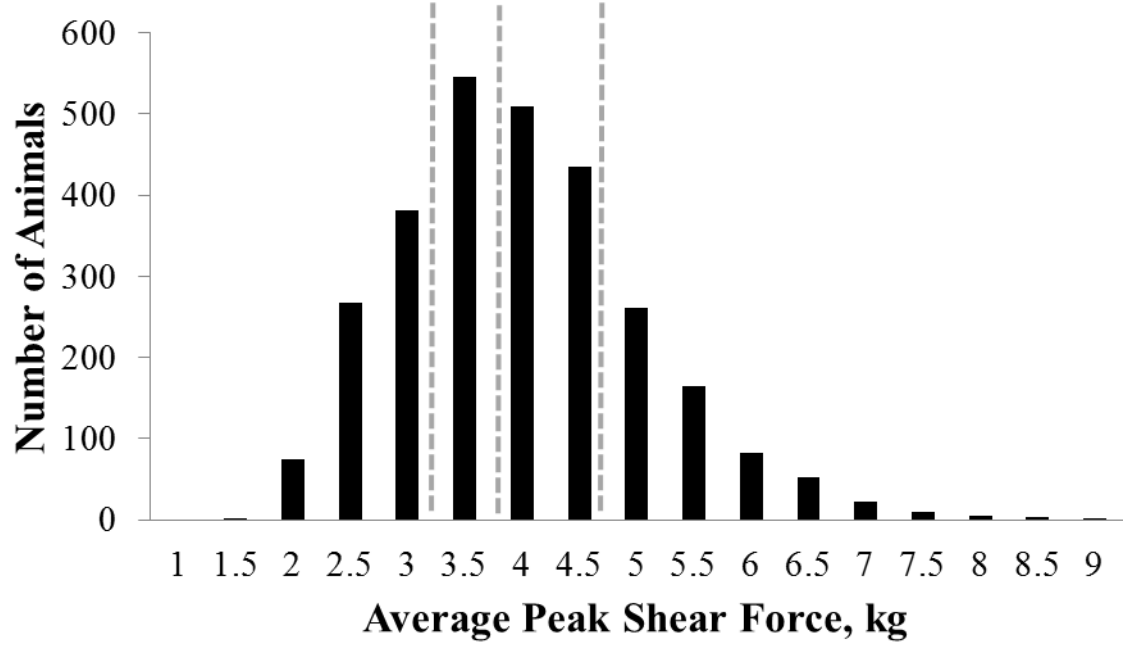
Model <sup>1</sup>	Log-likelihood	Number of Variance Parameters Estimated	Residual Degrees of Freedom	BIC
1	-6,662.71	12	4,313	13,369.04
2	-6,575.00	7	4,396	13,175.50
3	-3,703.94	7	10,660	7,436.07
4	-2,754.44	6	2,904	5,529.66

<sup>1</sup> Model 1 (n = 3,042) was a tri-variate animal model including APSF, EV1, and EV2.

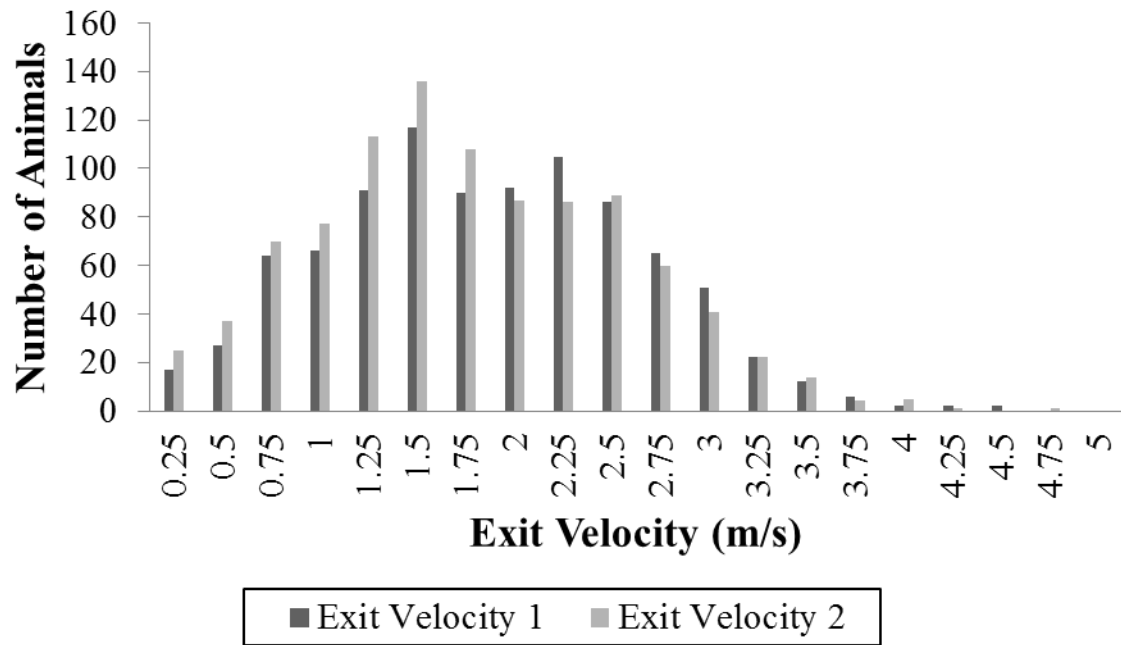
Model 2 (n = 3,042) analyzed APSF and exit velocity as a repeated record of EV1 and EV2.

Model 3 (n = 1,871) analyzed both WBSF and EV as repeated record.

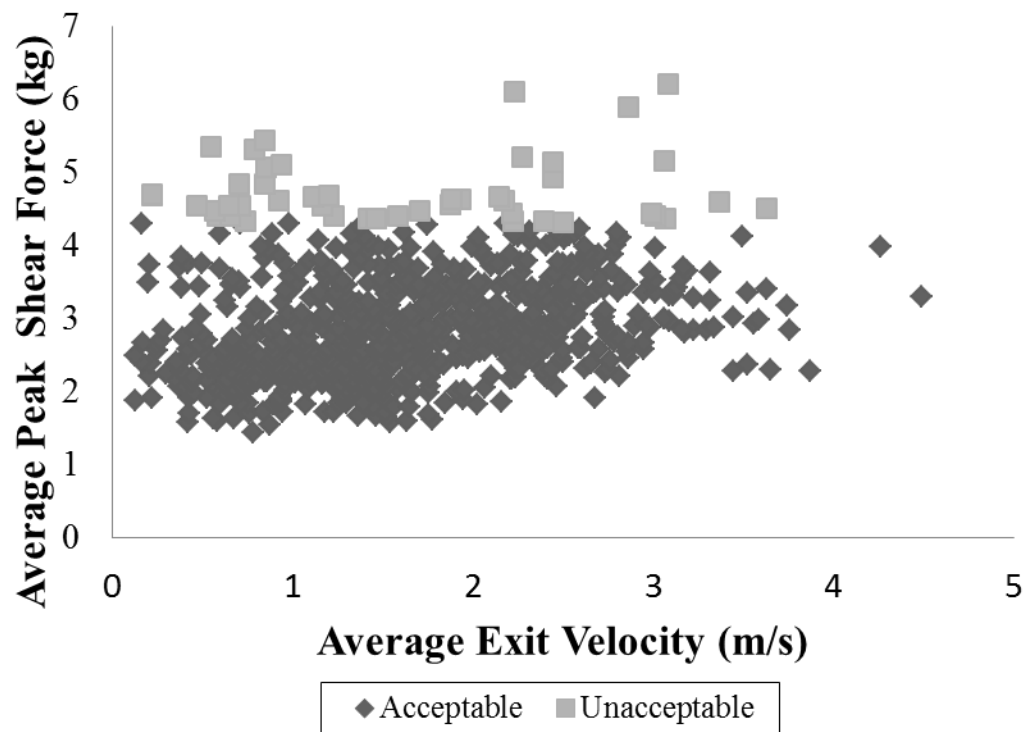
Model 4 (n = 1,871) analyzed APSF and EV as a repeated record.



**Figure 2.1.** Histogram of average peak shear force (APSF) demonstrating the normal distribution of the phenotypic observations in the data. The dotted gray lines indicate very tender (< 3.2-kg), tender (3.2-kg to 3.9-kg), intermediate (3.9-kg to 4.6-kg), and tough (> 4.6-kg) steaks according to Savell et al. (2006).



**Figure 2.2.** Histogram of exit velocity 1 (EV1) and exit velocity 2 (EV2) demonstrating the normal distribution of the phenotypic observation of EV1 and EV2 in the data. Exit velocity 1 was measured on the first day of a feeding trial and the EV2 measurement was taken 42 days later.



**Figure 2.3.** Mean phenotypic exit velocity (EV) by average peak shear force (APSF) phenotype illustrating the distribution of acceptable and unacceptable steaks across a range of average EV observations.

## **CHAPTER 3**

### **GWAS FOR TEMPERAMENT AND TENDERNESS**

#### **TRAITS**

##### **Summary**

A genome-wide association study (GWAS) was conducted for temperament and beef tenderness traits utilizing genotypes generated by the Illumina BovineSNP50 BeadArray (San Diego, CA, USA; Matikumalli et al., 2009) to identify single-nucleotide polymorphism (SNP) markers associated with phenotypic variation in exit velocity (EV; temperament) and average peak shear force (APSF; tenderness) measurements. Two Bayesian statistic models were used to estimate the proportion of markers, heritability, and accuracy of SNP prediction equations (estimated as correlations between predicted and true breeding value) developed in randomly assigned training populations. A three-fold cross validation procedure was utilized for development of prediction equations using training and validation populations. Bayes-C assumed that all SNP markers were drawn from a population of markers with the same variance, and used all available markers in the analysis. Bayes-C $\pi$  assumed independent variances for each SNP marker

with estimates of variance produced for the  $(1 - \pi)$  proportion of markers with significant effects. The remaining proportion,  $\pi$ , was assumed to have no effect on phenotype.

Temperament was measured as EV and recorded via two methods; (1) a single measurement was analyzed as a phenotype and (2) the second as a difference between two repeated EV measurements, spaced over 42 days, to analyze habituation to handling. The GWAS revealed that for both EV and habituation, 2 (0.006%) of SNP markers from the Illumina BovineSNP50 BeadArray contributed significantly to the additive genetic variance for these traits. Heritability estimates varied between the two Bayesian models. The EV heritability  $\pm$  standard error estimates were  $0.23 \pm 0.0003$  for the Bayes-C model and  $0.04 \pm 0.00008$  for Bayes-C $\pi$  model. Habituation heritability  $\pm$  standard error estimate was  $0.10 \pm 0.0001$  and  $0.02 \pm 0.00006$  for the Bayes-C and Bayes-C $\pi$  models, respectively. The accuracy of molecular breeding value (MBV) predictions developed during the training phase and evaluated in the validation dataset for EV averaged 0.45 (Bayes-C $\pi$ ), and near zero for habituation. Tenderness was measured by Warner-Bratzler shear force (WBSF), and reported as APSF, the mean of WBSF produced on multiple steak core samples. The variation in APSF was associated with 70 (0.167%) of the markers on the Illumina BovineSNP50 BeadArray. Heritabilities for APSF were similar between the two Bayesian models;  $0.15 \pm 0.002$  (Bayes-C),  $0.13 \pm 0.0002$  (Bayes-C $\pi$ ). Accuracy of MBVs for APSF was 0.27 in the three-fold validation. Correlations for both EV and APSF were 0.10, and the correlation for habituation was zero.

## Introduction

The Illumina BovineSNP50 BeadArray (San Diego, CA, USA; Matikumalli et al., 2009) has been used by researchers to conduct genome-wide association studies (GWAS) for a variety of traits in cattle. Cattle temperament is an often over looked trait for genetic improvement. Docile temperaments can be beneficial to a production system. Calmer animals adapt more easily and become less stressed with repeated handling as well as pose fewer problems to handlers, farm equipment, and the animal's safety (Grandin, 1997). Exit velocity, first explored by Burrow et al. (1988) is a reliable measure of temperament. Beef tenderness, measured as Warner-Bratzler Shear Force, has been evaluated across breeds and institutions. Beef tenderness influences beef quality, palatability, and overall eating satisfaction, so much so that consumers are willing to pay a premium for a tender steak (Lusk et al., 2001; Platter et al., 2005; McClure et al., in press).

To allow beef producers to efficiently select for improvement in temperament or tenderness using genetic markers, GWAS must be reliable and accurately predict the genetic variation in the trait of interest. The primary objective of this study was to conduct a GWAS to detect single-nucleotide polymorphism markers associated with temperament or tenderness in Simmental  $\times$  Angus crossbred cattle.



## Materials and Methods

The animals ( $n = 3,042$ ) used in Chapter 2 were reduced 1,432 animals on the basis of the availability of at least one EV observation and/or WBSF observations for each steak core. Animals were chosen for genotyping to represent an even distribution of WBSF values. All animals were progeny of Simmental sires and Angus dams.

Temperament was measured as exit velocity (EV). Exit velocity is the rate (m/s) at which animals exit the working chute and cover a distance of 1.7-m (Burrow et al., 1988; Curley et al., 2006). The elapsed time is recorded in hundredths of a second by a simple electronic system. Two light beams are focused on infra-red sensors spaced at the chute (head bail) and 1.7-m away from the head bail. The sensors have an on/off mechanism, so that as the animal breaks the light beam, the timing apparatus stops and a connected computer records the time (Burrow et al., 1988; Curley et al., 2006; Beckman et al., 2007). The elapsed time required for the animal to pass between the two sets of infra-red sensors is recorded, and is termed the animal's flight time (FT). Exit velocity is computed as the distance traversed divided by FT and is reported as m/s (Beckman et al., 2007). Each animal had the potential to have two EVs, one (EV1) indicating day one, and the second (EV2) was measured on day 42 of the feeding period.

The protocol used for all Warner-Bratzler shear force (WBSF) measurements followed the protocol outlined by Dikeman et al. (2005), which closely follows all of the American Meat Science Association (AMSA) recommendations as well as the proposed shear force procedures for meat tenderness measurements (Wheeler et al., 2005). The *longissimus dorsi* (LD) muscle was collected at the slaughter plant, and once returned to

the research institution 2.54-cm steaks were cut and immediately vacuum-packaged. The steaks were aged at 2°C for 14 days, thawed for 24 hours, and then then cooked on a convection conveyor oven (XLT Oven Model 1832-EL, BOFI, Inc., Wichita, KS) to an internal temperature of 71°C (medium degree of doneness). After the steaks were taken from the conveyor oven, internal temperatures were measured with a hand-held thermometer with a wire thermocouple (HH-21, Omega Engineering, Stamford, CT USA), and after the post-cooking temperature rise was complete, this temperature was recorded in order to adjust the WBSF measurement. The steaks were chilled at 2°C for 24 hours and an average of eight 1.27-cm steak cores were removed parallel to the muscle fiber orientation using a hand-held coring device. Each core was then sheared using a United-Smart 1 Test System SSTM-500 (United Calibration Crop., Huntington Beach, CA) with a head speed of 250-mm/min, and shear force was recorded. Core shear force values were averaged and reported as average peak shear force (APSF), which was used in the analysis.

All DNA samples were genotyped at GeneSeek with the Illumina BovineSNP50 BeadArray (San Diego, CA, USA; Matikumalli et al., 2009) for 54,790 SNPs. The resulting genotypes were then filtered to remove markers with a minor allele frequency < 0.05, or were not mapped to chromosomes on the University of Maryland sequence assembly (UMD3.1; Zimin et al., 2009). Subsets of the samples were genotyped on versions 1 and 2 of the Illumina BovineSNP50 BeadArray and markers present on both versions of the array were extracted for use in the GWAS. Genotypes were processed through FastPHASE v 1.4.0 (FastPHASE; Scheet and Stephens, 2006) to

estimate haplotypes and missing genotypes. The number of clusters was set to 20, and the command used was *fastPHASE -T10 -K20 -eo -oFP\_0101.OUT fastphase0101.inp*.

After editing the genotypes, the dataset contained 42,351 SNPs for analysis.

Phenotype files were created to analyze all traits. Two EV phenotype files were created. Results from the Chapter 2, which includes the animals in this study, estimated a genetic correlation of  $0.99 \pm 0.07$ , and the phenotypic variance was similar between EV1 and EV2. The records for EV1 ranged from 0.07 to 4.48-m/s and 0.09 to 4.63-m/s for EV2. The mean for both EV1 and EV2 was 1.70-m/s. Therefore, the first EV phenotype used EV1 if it was present. If EV1 was not recorded, EV2 was used, and animals without any EV measurement were deleted from the temperament phenotype file. This EV phenotype file contained 734 animals. The variation in this phenotype file was used to associate temperament with quantitative trait loci (QTL) in the genome. The second temperament file was created from animals that had both EV1 and EV2 measurements. Exit velocity 2 was subtracted from EV1 to produce a habituation score, and the file contained 587 animals. The variation in this phenotype file was used to associate habituation to human handling to QTLs in the genome. An APSF phenotype file was created which contained 1,096 animals. Variation in APSF was associated with QTLs in the genome. Uniform contemporary groups (CG) were assigned based on common year of birth and herd of origin. Contemporary groups including a single animal were removed from analysis, resulting in 599, 575, and 957 animals in the EV, habituation, and APSF phenotype files, respectively.

The phenotype and genotype files were uploaded onto the Bioinformatics to Implement Genomic Selection (BIGS) Project website (<http://big.s.ansci.iastate.edu/>) to utilize the GenSel software (Fernando and Garrick, 2008). Bayesian estimation procedures were then used to estimate single-nucleotide polymorphism (SNP) marker effects on each of the traits.

The general statistical model for both the Bayes-C and Bayes-C $\pi$  GWAS procedures was:

$$y = X\beta + \sum_{j=1}^K z_{ij}\alpha_j \delta_j + e_i$$

where  $y$  is the vector of phenotypes,  $X$  is an incidence matrix relating animals to fixed effects to be estimated in  $\beta$ ,  $K$  is the number of SNPs,  $z_{ij}$  is the covariate at locus  $j$  for individual  $i$ ,  $\alpha_j$  is the random allele substitution effect for locus  $j$ , which is conditional on  $\sigma_a^2$  and is assumed normally distributed when  $\delta_j = 1$  but  $\alpha_j = 0$  when  $\delta_j = 0$ ,  $\delta_j$  is a random 0/1 variable indicating the absence or presence of locus  $j$  in the model, and  $e$  is a vector of residual effects (Habier et al., 2011; Kizilkaya et al., 2011).

The Bayes-C model assumes a constant SNP marker variance for all SNP markers and a known  $\pi$ . When the model is fit with  $\pi = 0$ , it is equivalent to a genomic best linear unbiased prediction (G-BLUP) model, in which all SNP markers have non-zero allele substitution effects estimated (Garrick et al., 2010; Habier et al., 2011). The Bayes-C $\pi$  model assumes a constant SNP marker variance for the SNP markers that are included into the model and estimates  $\pi$ , which is used to shrink unassociated SNP marker effects to zero (Garrick et al., 2010; Habier et al., 2011).

All phenotypes were analyzed using both the Bayes-C and Bayes-C $\pi$  methods, and contained CG as a fixed effect. Five analyses under both Bayesian models for each phenotype were completed using various random number seeds to initialize the Markov chains. The random number seed affects the SNP markers that are allocated at random to the model to test their effects. A total of 160,000 Markov chain Monte Carlo (MCMC) iterations, with a burn-in of 1,000 iterations, were completed for each analysis. Results from each analysis included posterior distributions for the effects of each of the 42,351 SNP markers, and posterior means for the fixed effects which were used to correct the trait values to phenotypes before the training and validation processes were executed. The resulting phenotype file is corrected for fixed effects and mean zero. The animals in the adjusted phenotype files were then randomly subdivided into thirds. Model training was done in two-thirds of the data, and cross validation in the other third. Training and cross validation was completed in five different random subdivisions of animals for each phenotype. By changing the random number seed and reallocating animals into different training and validation populations, the chance of Type-I errors are reduced, making it less likely for a false discovery of associated SNP markers. The random number seed affects the SNP markers that are allocated to the model at random to test their effect. By subdividing the training and validation populations, the phenotypic variance is partitioned differently. Only SNP markers with true large effects will be associated with the variation in the trait in each analysis.

Accuracy of the training group molecular breeding value model predictions was estimated in the validation populations as:

$$Accuracy = \frac{corr(\hat{g}, y)}{\sqrt{h^2}}$$

where ***corr*** ( $\hat{g}, y$ ) is the estimated correlation between predicted breeding values and observations (provided in GenSel output), and  $h^2$  is the heritability estimate.

The markers with the largest effects that are included in the prediction model allow the estimation of  $(1 - \pi)$  for EV, habituation, and APSF from the Bayes-C $\pi$  analysis. Regions of the genome harboring the markers that were consistently included in the prediction model across all analyses were queried using the UCSC Genome Browser and NCBI Entrez Map Viewer to identify potential candidate genes for temperament, habituation, and tenderness. Both browsers (UCSC and NCBI) were used to take advantage of the Baylor (4.0) and Maryland (3.1) genome assemblies.

## Results and Discussion

### GWAS for EV and Habituation

Observations in EV ranged from 0.07 to 4.26-m/s and had an average of  $1.80 \pm 0.83$ -m/s. Chapter 2 contained 399 more EV observations (between EV1 and EV2) which ranged from 0.07 to 4.63-m/s with an average in both EV1 and EV2 of  $1.70 \pm 0.76$ -m/s. The habituation observations ranged from -2.32 to 4.76-m/s and averaged -0.15-m/s. Table 3.1 reports the average posterior means for variance components estimated by the Bayes-C and Bayes-C $\pi$  models for the EV and habituation phenotypes. The Bayes-C $\pi$  model results were assessed for EV, and 2 (0.006%) of the 42,351 markers were associated with variation in the EV phenotype. The 2 (0.006%) markers explained a fairly low estimated heritability ( $0.04 \pm 0.0008$ ) for EV, as opposed a moderate heritability ( $0.23 \pm 0.0003$ ) when all markers were included via the Bayes-C model. Chapter 2 consisted of a larger group ( $n = 3,029$ ) of animals from which the animals in this study were derived ( $n = 599$ ). The heritability estimate of  $0.39 \pm 0.09$  for EV was recorded in Chapter 2. The decrease in heritability estimates is a direct reflection of having fewer animals in the analysis. Results from the two studies support the conclusion that EV is moderate to lowly heritable, verifying that EV is extremely environmentally influenced. In other studies, the heritability of EV has been estimated to be of 0.35 among *Bos taurus* breeds (Burrow and Corbet, 2000; Beckman et al., 2007). Other studies have found similar heritability estimates for measurements of temperament using EV; 0.49 in *Bos taurus* breed crosses (Nkrumah et al., 2007) and 0.31 in *Bos taurus* and *Bos indicus* breed crosses (Johnston et al., 2003a; Reverter et al., 2003; Kadel et al., 2006). The habituation

phenotype had a low estimated heritability, which is expected due to the genetic correlation of 0.99 seen in Chapter 2. The estimated heritability utilizing all of the markers (Bayes-C) was  $0.10 \pm 0.0001$ , while the Bayes-C $\pi$  analysis estimated that only 2 (0.006%) of the markers had significant effects and yielded a heritability of  $0.02 \pm 0.00006$ . Accuracy of the training group prediction on the validation populations for EV ranged from 0.05 to 0.94, with a mean of 0.45, and are also shown in Table 3.2. The low average accuracy is a direct reflection of having too few animals in the analysis. Habituation accuracies are shown in Table 3.3. The accuracy from training and validation populations are near zero.

The EV phenotype resulted in 2 (0.006%) of the 42,351 markers being associated with the phenotypic variance. The 2 markers most commonly associated with EV were located on BTA10 (UMD3.1 location 88,813,187 bp) and BTA12 (UMD3.1 location 30,967,371 bp). The habituation phenotype resulted in 2 (0.006%) of the 42,351 markers being associated with the phenotypic variance. The SNP markers with the largest effect on the habituation phenotype data were mapped to BTA8 (UMD3.1 location 82,922,937 bp) and BTA12 (UMD3.1 location 59,185,443). None of the markers were consistently identified during analysis of all training populations, and there was no overlap of markers between the EV and habituation phenotypes. This result indicates the dataset was too small to consistently identify even the largest effect QTL in EV and habituation.



## GWAS for APSF

Observations in APSF ranged from 1.58 to 8.36-kg and averaged  $3.76 \pm 1.15$ -kg. Chapter 2 contained 67 more observations for APSF which ranged from 1.43 to 8.86-kg with an average of  $3.46 \pm 1.42$ -kg. Approximately 70 (0.167%) of the 42,351 makers were found to be associated with variation in APSF. The SNP markers with the largest effect yielded an estimated heritability of  $0.13 \pm 0.0002$ , whereas utilizing all the SNP markers (Bayes-C model) produced an estimated heritability of  $0.15 \pm 0.0002$ , as shown in Table 3.1. This study consisted of a subset of animals from Chapter 2 that were selected to represent WBSF observations across the phenotypic distribution. Heritability estimates were  $0.19 \pm 0.06$  in APSF of 3,042 animals. When 1,871 animals were in the analysis, estimated heritability was  $0.06 \pm 0.05$ . The similar heritability estimates between the 3,042 animals and the GWAS suggest similar estimates of additive genetic variance. A low heritability indicates that the trait is highly influenced by environment, and therefore low SNP marker effects are predicted. In other studies, APSF estimates of heritability in Angus-sired steers was  $0.33 \pm 0.25$  and in Simmental-sired steers was  $0.16 \pm 0.14$  (Minick et al., 2004). The correlations between true and predicted breeding values and corresponding accuracies determined in the validation populations, in Table 3.4, are low. Accuracies ranged from 0.10 to 0.76, and an average accuracy of 0.27 was estimated.

For APSF, 70 (0.167%) of the 42,351 markers were associated with the phenotype. The 70 markers most commonly associated with APSF were located on all the bovine chromosomes except 18, 20, 24, 26, 28, and X. A summary of the chromosomes

and marker locations are shown in Table 3.5. None of the markers were consistently identified in all training populations. This result suggests that there was insufficient data to consistently identify an effective QTL for APSF and that the SNP markers identified maybe spurious. *CAST* (BTA7) and *CAPNI* (BTA29) are the two most documented genes with significant effect on WBSF, and were not found in the list of markers. This may be because the number of animals in the analysis was too small to find an effect. Alternatively, the animals in this analysis maybe fixed or have low variation in haplotypes in the regions of these two genes. McClure et al. (in press) reports that the *CAST* SNP included in the Illumina BovineSNP50 BeadArray explained a small percentage (0.02%) of APSF phenotypic variation in Simmental cattle. The variation in phenotype explained by the panel was subsequently lower in Simmental and Angus than other breeds.

## Conclusions

Like many studies in this area, the relatively small number of animals genotyped substantially limits the ability of GWAS to identify genomic regions harboring QTL that play major roles phenotypic variation. Results in Chapter 2, which included the pedigree of these animals, reported a heritability estimate of temperament, when measured as an EV, to be 0.39. The GWAS analysis, using fewer phenotypic records on a subset of animals from Chapter 2, estimated the heritability to be 0.23. The estimate of APSF heritability reported in Chapter 2 was 0.19, which is similar to the 0.15 heritability estimates for APSF in this study. A recent study by McClure et al. (in press) estimated the heritability of APSF in 516 Simmental animals to be 0.08 and in 651 Angus animals to be 0.52. Information on these animals were also part of the Carcass Merit Project. Accuracies of molecular breeding values are critical parameters for implementation of genomic selection. Improved accuracies are required before any SNP markers on the Illumina BovineSNP50 BeadArray are considered for selection to improve EV or APSF.

Due to the small sample size only 2 (0.006%) of the markers were found to associate with the variation in EV, and 2 (0.006%) markers were associated with the variation in habituation; however, 70 markers (0.167%) were associated with variation in tenderness. Insufficient phenotypes were available to consistently identify QTL regions underlying these traits. Similar additive genetic variance is seen in the APSF data between Chapter 2 and this study, suggesting similar results if more phenotypes were available.

**Table 3.1.** Average posterior means of genetic variance, residual variance, heritability, and percent of SNP markers selected in the Bayes-C and Bayes-C $\pi$  analyses of exit velocity (EV), habituation, and average peak shear force (APSF).

Trait	Bayesian Model	No. of Animals	Genetic variance	Residual variance	Heritability	Percent of markers in analysis <sup>2</sup>
EV	Bayes-C	599	0.11 $\pm$ 0.00010	0.35 $\pm$ 0.00013	0.23 $\pm$ 0.00028	100 <sup>5</sup>
EV	Bayes-C $\pi$	599	0.02 $\pm$ 0.00004	0.44 $\pm$ 0.00007	0.04 $\pm$ 0.00008	0.006
Habituation <sup>1</sup>	Bayes-C	575	0.04 $\pm$ 0.00017	0.41 $\pm$ 0.00008	0.10 $\pm$ 0.00011	100 <sup>5</sup>
Habituation <sup>1</sup>	Bayes-C $\pi$	575	0.01 $\pm$ 0.00003	0.44 $\pm$ 0.00007	0.02 $\pm$ 0.00006	0.006
APSF	Bayes-C	957	8.43 $\pm$ 0.01	46.37 $\pm$ 0.01	0.15 $\pm$ 0.002	100 <sup>5</sup>
APSF	Bayes-C $\pi$	957	7.25 $\pm$ 0.009	38.41 $\pm$ 0.01	0.13 $\pm$ 0.0002	0.167

<sup>1</sup> Habituation is estimated by the difference between exit velocity 1 (EV1) and exit velocity 2 (EV2) which are measured 42 days apart.

<sup>2</sup> The percent markers used in the analysis was calculated by  $[(1 - \pi) \times 100]$ .

**Table 3.2.** Accuracies of molecular breeding values (MBV) produced in all three-fold cross validation GWAS for the exit velocity (EV) phenotype in the Bayes-C $\pi$  analysis.

	Replicate <sup>2</sup> 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
Populations <sup>1</sup>	Accuracy <sup>3</sup>				
Train in 1 and 2, Validate in 3	0.10	0.07	0.62	0.25	0.61
Train in 1 and 3, Validate in 2	0.39	0.66	0.94	0.68	0.05
Train in 2 and 3, Validate in 1	0.06	0.82	0.23	0.70	0.50
<b>Overall Mean</b>	<b>0.45</b>				

<sup>1</sup> The total population was randomly divided into thirds for the EV (n =734) dataset and formed into groups 1, 2, and 3. The numbers following the “Train in” and “Validate in” in the table denote which groups were contained in the training and validation populations.

<sup>2</sup> The EV dataset was randomly divided into one-thirds five separate times. The columns in the table, labeled as replicate, represent these separate partitionings.

<sup>3</sup> Accuracies were calculated as  $\frac{corr(\hat{g}, y)}{\sqrt{h^2}}$ .

**Table 3.3.** Accuracies of molecular breeding values (MBV) produced in all three-fold cross validation GWAS for the habituation phenotype in the Bayes-C $\pi$  analysis.

	Replicate <sup>2</sup> 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
Populations <sup>1</sup>	Accuracy <sup>3</sup>				
Train in 1 and 2, Validate in 3	-0.05	-0.25	-0.10	0.75	-0.29
Train in 1 and 3, Validate in 2	-0.09	0.46	0.52	0.43	-0.32
Train in 2 and 3, Validate in 1	0.02	-0.41	-0.05	0.29	0.20
<b>Overall Mean</b>	<b>0.07</b>				

<sup>1</sup> The total population was randomly divided into thirds for the EV (n =587) dataset and formed into groups 1, 2, and 3. The numbers following the “Train in” and “Validate in” in the table denote which groups were contained in the training and validation populations.

<sup>2</sup> The EV dataset was randomly divided into one-thirds five separate times. The columns in the table, labeled as replicate, represent these separate partitionings.

<sup>3</sup> Accuracies were calculated as  $\frac{corr(\hat{g}, y)}{\sqrt{h^2}}$ .

**Table 3.4.** Accuracies of molecular breeding values (MBV) produced in all three-fold cross validation GWAS for average peak shear force (APSF) phenotype in the Bayes-C $\pi$  analysis.

	Replicate <sup>2</sup> 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
Populations <sup>1</sup>	Accuracy <sup>3</sup>				
Train in 1 and 2, Validate in 3	0.18	0.14	0.76	0.15	0.27
Train in 1 and 3, Validate in 2	0.16	0.12	0.10	0.15	0.24
Train in 2 and 3, Validate in 1	0.26	0.62	0.21	0.31	0.30
<b>Overall Mean</b>	<b>0.27</b>				

<sup>1</sup> The total population was randomly divided into thirds for the APSF (n =957) dataset and formed into groups 1, 2, and 3. The numbers following the “Train in” and “Validate in” in the table denote which groups were contained in the training and validation populations.

<sup>2</sup> The EV dataset was randomly divided into one-thirds five separate times. The columns in the table, labeled as replicate, represent these separate partitionings.

<sup>3</sup> Accuracies were calculated as  $\frac{corr(\hat{g}, y)}{\sqrt{h^2}}$ .

**Table 3.5** Chromosome number and location of the most commonly detected single-nucleotide polymorphism (SNP) markers associated with average peak shear force (APSF).

Chromosome <sup>1</sup>	Location (Mbp) <sup>2</sup>
1	7.63, 20.81, 20.85, 65.28, 111.96, 116.05, 126.37, 129.12, 129.17, 143.62, and 158.23
2	19.16, 37.07, 79.16, 79.18, 89.75, 89.78, and 135.67
3	11.13, 90.33, and 107.96
4	36.56, 55.75, 59.71, 92.74, 92.77, and 109.29
5	40.49 and 44.11
6	11.12
7	13.34 and 39.68
8	15.42, 16.73, and 77.58
9	22.89, 25.41, 82.08, 82.18, and 82.90
10	46.35, 68.53, and 98.54
11	66.53, 67.02, and 67.06
12	52.46
13	25.82 and 73.65
14	62.29
15	67.16
16	3.26 and 38.63
17	65.32
19	10.22, 35.62, and 60.27
21	10.90 and 53.63
22	12.48, 14.38, and 20.72
23	27.06 and 34.12
25	3.26, 31.67, and 42.34
27	6.87 and 24.46
29	34.36

<sup>1</sup>Chromosome is the same for both Baylor (4.0) and Maryland (3.1) assemblies for all listed markers.

<sup>2</sup>SNP location is based on the University of Maryland 3.1 assembly and listed in mega base pairs (Mbp).



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