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A QUANTITATIVE AND MOLECULAR EVALUATION OF CALVES SIRED BY THREE
DIFFERENT BREEDS FOR GROWTH, PERFORMANCE, AND CARCASS TRAITS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Animal Sciences

by
Malcolm Seth Mizell
B.S., Louisiana State University, 2011
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ABSTRACT

The first objective of the two studies in the research herein was to evaluate growth, performance and carcass quality and composition traits from a group of spring born calves sired by Charolais, Braunvieh, or Simmental bulls. Growth traits analyzed included birth weight (BW), weaning weight (WW), and hip height (HH). Carcass quality and composition traits analyzed included hot carcass weight (HCW), rib eye area (REA), back fat thickness (BF), marbling score (MS), and yield grade (YG). Analysis revealed Simmental sired calves were significantly heavier ($P<.05$) at birth when compared to Charolais and Braunvieh sired calves. Braunvieh sired calves were reported to be significantly ($P<.05$) shorter than Simmental sired calves for hip height. Hot carcass weight analysis revealed that Charolais sired calves were significantly ($P<.05$) heavier when compared to Braunvieh sired calves. No individual sire breed group displayed any significant differences for weaning weight, rib eye area, back fat thickness, marbling score, or yield grade.

The second objective was evaluate potential SNP associations on four candidate genes with growth, performance, and carcass composition traits in a population of crossbred steers sired by Charolais, Braunvieh, and Simmental bulls. Traits analyzed for growth, performance, and carcass traits were previously mentioned. Analysis was performed on 38 steer calves. Single nucleotide polymorphisms within four candidate genes including Thyroglobulin (TG), Adiponectin (ADPOQ), Insulin like growth factor-1 (IGF-1), and Calpastatin (CAST) were chosen for analysis. A total of 14 SNP on TG, ADPOQ, CAST, and IGF-1 genes were significantly associated with birth weight and weaning weight. Analysis revealed 13 SNP on TG, ADPOQ, IGF-1, and CAST genes significantly associated ($P<.05$) with HCW, BF, MS, and YG. No markers were reported

to be significantly associated ($P < .05$) with HH or REA. A total of 11 markers with 3 markers located on the ADPOQ gene, 1 marker located on the IGF-1 gene, 3 markers located on the TG gene, and 5 markers located on the CAST gene were significantly associated with both growth and carcass traits.

CHAPTER I. INTRODUCTION

The majority of cattle that comprise the beef cattle industry in the Southeastern United States are crossbred animals rather than purebred animals (Coleman et al., 2012). Producers' in a crossbreeding program can utilize breeds that exhibit traits of importance in respect to the environment their cattle are raised. Crossbreeding between *Bos taurus* and *Bos indicus* cattle has been widely practiced in subtropical regions due to the benefit of heterosis and breed complementarities for reproduction, growth, and carcass quality traits (Kim et al., 2003). It is through crossbreeding where breeds such as the Brahman are utilized to achieve offspring predisposed to heat tolerance and parasite resistance. Knowledge of breed composition can be a beneficial tool when selecting cattle for various areas of production (Kuehn et al., 2011). There are many categories for which producers can choose to market their cattle. In any area chosen growth traits and carcass quality and composition traits are of great importance as they have an effect on profit margins. The research presented herein is an evaluation of growth, performance and carcass quality and composition traits from a group of spring born calves sired by Charolais, Braunvieh, or Simmental bulls. Growth traits included birth weight (BW), weaning weight (WW), and hip height (HH). Carcass quality and composition traits included hot carcass weight (HCW), back fat thickness (BF), rib eye area (REA), marbling score (MS), and yield grade (YG).

During the past few decades, advances in molecular marker based research have led to the identification of multiple genes or genetic markers associated with genes that affect traits of economic importance in livestock (Dekkers, 2004). Prior to genetic based selection, phenotypic selection was the primary resource for sire or dam

selection. A molecular marker is based on an animal's own individual nucleotide sequence mutation within their respective genome (Wanjie et al., 2013). More specifically, single nucleotide polymorphisms that are sequence polymorphism caused by a single nucleotide mutation which results in a base pair alteration at a specific locus on a DNA sequence (Wanji et al., 2013). Previously reported SNP for the IGF-1 and CAST genes were utilized for potential SNP associations with growth, performance, and carcass composition in a population of crossbred steers sired by Charolais, Braunvieh, and Simmental bulls while new SNP were utilized for the TG and APOQ genes. Several fetal and adult tissues synthesize IGF-1 which also controls growth and differentiation while being expressed by the liver (Bauman, 1992). Thyroglobulin is the precursor to various thyroid hormones with known endocrine roles in fat metabolism (Barendse, 1999) and has been previously included in association analysis test for carcass quality and composition traits by Casas et al., (2005). Adiponectin has been previously reported to modulate several biological processes including energy homeostasis, glucose, and lipid metabolism while being the most abundant protein secreted exclusively by the white adipose tissue in several mammalian species (Shin et al., 2013). The CAST gene is involved in the calpain-calpastatin enzyme complex that has been shown to regulate the rate of protein degradation by calpain activity accelerating protein degradation and calpastatin activity inhibiting this process (Koohmaraie et al., 2002).

CHAPTER II. REVIEW OF LITERATURE

Heterosis

Heterosis or hybrid vigor is defined by the performance of crossbred offspring excelling in performance at a greater level when compared to the respective purebred parents (Greiner, 2009). The majority of cattle that comprise the beef cattle industry in the Southeastern United States are crossbred animals rather than purebred animals (Coleman et al., 2012). Crossbreeding between *Bos taurus* and *Bos indicus* cattle has been widely practiced in subtropical regions due to the benefit of heterosis and breed complementarities for reproduction, growth, and carcass quality traits (Kim et al., 2003). Producers can crossbreed two separate breeds that display different traits of interest in the idea that subsequent offspring will express traits of interest from each parent. The advantage of crossbreeding cattle has been well documented (Gray et al., 1978) and records indicate an increased performance in an assortment of traits such as performance and carcass traits. Koger and associates (1975) reported higher levels of heterosis from crosses of *Bos indicus* breeds with *Bos Taurus* breeds. Utilizing hybrid vigor to ensure maximum productivity is an advantageous system with the ideal cow being one that first calves at 2 years old, maintains a 365-day calving interval, while having no human intervention, and weaning a marketable calf. Utilizing diverse breeds allows researchers to delve into heterosis and complementarities through crossbreeding, which would allow producers to select schemes that are a representation of their area of production (Wheeler et al., 2001). The issues that arise for producers instituting the aforementioned breeding scheme are the lack of genetic

research conducted that estimates genetic expression in crossbred populations. Knowledge of breed composition can be a beneficial tool when selecting cattle for various areas of production (Kuehn et al., 2011).

A multi-breed evaluation that includes all crossbred and purebred individuals into a single analysis has been proposed as an alternative (Arnold et al., 1992). This type of evaluation would allow all progeny of an individual to influence its performance evaluation (Klei et al., 1996) and presumably lead to greater accuracies and less conflicting associations. In a more recent study, Kuehn & associates, (2011) reported results indicating that breed frequencies predicted from high density SNP panel could be used to predict breed composition of crossbred animals.

MARC Germplasm Studies

The United States Department of Agriculture Meat Animal Research Center (MARC) in Clay Center, Nebraska has routinely conducted studies where the objective is to evaluate specific cattle breed performances. These studies evaluate several breeds and analyze cattle at various stages of the production scheme (Thallman et al., 1999). Germplasm evaluation IV included the traits of post-weaning growth, puberty, and pregnancy traits on heifers sired by Angus, Hereford, Charolais, Shorthorn, Galloway, Longhorn, Nellore, Piedmontese, and Saler bulls crossed with Hereford and Angus dams (Thallman et al., 1999). Piedmontese sired heifers were significantly associated with reaching puberty by 360 days and yielding the highest pregnancy rate when compared to all other breeds. It was further reported that the Piedmontese sires utilized in the study carried a mutation in the myostatin gene causing double-muscling (Casas et al., 1999; McPherron and Lee, 1997; Smith et al., 1997). The mutated

myostatin gene expression has previously been found to be associated with reduced fertility and delayed puberty (Arthur, 1995). The previous research on myostatin expression contradicts the results found in Cycle IV reproduction results. The researchers made the assumption that since the dam group of Hereford and Angus had a minimal probability of harboring the myostatin mutation that the female Piedmontese cross offspring were heterozygous for the mutated myostatin allele and there may be an over dominance for fertility at the myostatin locus (Thallman et al., 1999). In the area of growth traits the Hereford and Angus sired progeny were significantly heavier at weighing days 200, 400, and 550, and had significantly greater hip height at 550 days (Thallman et al., 1999).

The germplasm cycle V conducted by the U.S. Meat Animal Research Center characterized cattle breeds including two tropically adapted breeds with Brahman cattle, and two breeds with high frequencies of double muscling (Wheeler et al., 2001). Wheeler and associates (2001) evaluated double muscling, carcass quality and composition traits to analyze. There is definite need for research that analyzes breed differences in production efficiency and carcass quality simply due to the fact that no single breed excels in all traits. The breeds that Wheeler and associates (2001) evaluated were Hereford, Angus, and compositied generated from the MARC germplasm evaluation III cows bred to Hereford, Angus, Tuli, Boran, Brahman, Piedmontese, and Belgian Blue sires (Wheeler et al., 2001). Results indicated that no single breed excelled in every trait analyzed. Belgian Blue and Piedmontese sired calves excelled in yield grade and longissimus palatability. Hereford and Angus sired calves excelled in quality grade and longissimus palatability. The Tuli sired calves

excelled in alternative selection for heat tolerance with a subsequent negative effect on meat tenderness (Wheeler et al., 2001).

The germplasm cycle VI conducted by the U.S. Meat Animal Research Center characterized cattle that included Scandinavian breeds and Japanese Wagyu cattle (Wheeler et al., 2004). The breeds chosen to be evaluated were Hereford, Angus, and MARC III cows bred to Norwegian Red, Swedish Red, White Friesian, and Wagyu sires (Wheeler et al., 2004). Cycle VI of the germplasm program closely resembled cycle V, in which carcass quality and composition traits were evaluated. Wagyu sired calves rendered the highest percentage of USDA choice yield grade 1 and 2, greatest longissimus muscle tenderness, resulted in greater retail product compared to British breed, but were the smallest of sire breeds utilized (Wheeler et al., 2004).

The germplasm cycle VII conducted by the U.S. Meat Animal Research Center characterized cattle that would represent a current evaluation of the seven most prominent beef breeds in the United States; as compared to the evaluations recorded twenty-five to thirty years prior with sires from the same breeds excluding Red Angus (Wheeler et al., 2005). The breeds chosen to be evaluated were Angus, Hereford, Charolais, Limousin, Simmental, Red Angus, and Gelbvieh sires bred to MARC III dams (Wheeler et al., 2005). Carcass and longissimus palatability traits that affected carcass quality and composition were chosen for analyses. Results for this particular cycle were of great practical importance to cattle producers trying to select breed types for specific traits. Results indicated that European breeds were leaner, heavier muscled, and had higher yielding carcasses compared to British breeds but with significantly less marbling

than Angus or Red Angus. British breeds did show a significant increase in growth rate as compared to the studies from years past (Wheeler et al., 2005).

The germplasm cycle VIII conducted by the U.S. Meat Animal Research Center characterized a group alternative tropically adapted breeds' to tropically adapted breeds commonly utilized in the United States. (Wheeler et al., 2010). Wheeler and associates (2010) reported that diverse breeds are required to exploit heterosis. Prior studies reported that *Bos taurus* breeds crossed with *Bos indicus* breeds were exceptionally productive and efficient, especially in subtropical climates (Olson et al., 1991; Cundiff, 2005). However, when the proportion of *B. indicus* increased, the advantages of *B. indicus* crosses were hampered by older age at puberty and temperament (Turner, 1980; Thrift and Thrift, 2005) and an overall reduction in meat tenderness associated with the cross (Crouse et al., 1989). One objective of GPE cycle VIII program was to identify alternative tropically adapted germplasm that minimizes the detrimental traits of *B. indicus* breeds. The breeds chosen to be evaluated were Hereford, Angus, Brangus, Beefmaster, Bonsmara, and Romosinuano crossed with dam breeds of Angus and MARC III of which breed description was previously described in characterization of cattle cycle VII (Wheeler et al., 2010). Results reported that Angus offspring had significantly more tender longissimus muscle with Beefmaster having the least tender. Beefmaster and Brahman were reported to be heavier, fatter, less yielding, with similar marbling but less tender longissimus muscle than Bonsmara and Romosinuano. Angus offspring had significantly more tender longissimus muscle than the American composites Beefmaster and Brahman. Bonsmara and Romosinuano could both be considered for alternative tropically adapted breeds to incorporate into breeding

schemes having produced carcasses that are lighter, leaner, greater yielding, and having more tender longissimus muscle measurements than traditionally accepted Beefmaster and Brahman (Wheeler et al., 2010).

Genetic Marker

A molecular marker is based on an animal's own individual nucleotide sequence mutation within their respective genome (Wanjie et al., 2013). During the past few decades, advances in molecular marker based research have led to the identification of multiple genes or genetic markers associated with genes that affect traits of economic importance in livestock (Dekkers, 2004). Several molecular based markers are utilized in research including restriction fragment length polymorphisms and microsatellite DNA (Wanjie et al., 2013). The restriction fragment length polymorphism method was first established by Grodzicker & associates (1974) and is utilized to identify mutations among different individuals (Wanjie et al., 2013). Lonergan and associates (1995) utilized the RFLP approach on a group of crossbred animals and reported no significant associations with EcoRI and BamHI RFLP sites within the bovine calpastatin locus for variation in calpastatin activity postmortem.

Microsatellites DNA, also known as simple sequence repeats are common repetitive DNA sequences within eukaryotic genomes (Wanjie et al., 2013). Generally they consist of motifs which are made up of 1-6 base pair tandemly repeated several times throughout a DNA strand (Litt and Luty, 1989). In cattle production, microsatellites are most utilized in paternity testing of progeny as breeding is usually carried out on rangeland results in unknown sires for calving groups (Gomez-Raya et al., 2008). The use of single nucleotide polymorphisms has been proposed but microsatellites were

considered more reliable (Heaton et al., 2002). The repetitive nature and inheritance of microsatellite portions of DNA allows for genomic testing for carcass quality or performance traits. Stone and associates (1998) used six microsatellite markers to determine the presence or absence of the muscle hypertrophy locus (mh) allele in a group of half sibling families. Cattle inheriting the mutated mh allele had had increased rib eye area and birth weight compared to those not inheriting the mh allele (Stone et al., 1998)

QTL

Quantitative trait loci (QTL) are a chromosomal region of the genome containing genes or mutations affecting a quantitative trait with relatively large affect on said trait (Snelling et al., 2010). Genetic markers and linkage maps have provided the tools necessary to identify QTL associated with traits of economic importance (Stone et al., 1999; Casas et al., 2000; Casas et al., 2001; MacNeil and Grosz, 2002). Traits difficult or expensive to measure will benefit most from QTL research as portions harboring known mutations affecting these traits could serve as areas further studied through a candidate gene approach (Casas et al., 2003). Casas and associates (2003) reported QTL affecting birth weight, longissimus area, percent retail product, and marbling on chromosomes 5, 6, 9, 21, and 23. These reports are similar with results reported by (Keele et al., 1999; Stone et al., 1999) where QTL were associated with carcass composition and meat quality traits on chromosomes 1, 2, 5, and 13. It has been previously reported by Kim and associates (2003) that QTL affecting birth weight, yearling weight, and hot carcass weight were found on chromosome 2, 5, 6, 11, and 23.

The utilization of QTL as a genomic tool has the potential to improve selection for economical traits via marker-assisted selection (Andersson, 2001).

SNP

A single nucleotide polymorphism (SNP) is a sequence polymorphism caused by a single nucleotide mutation which results in a base pair alteration at a specific locus on a DNA sequence (Wanji et al., 2013). Single base transitions, transversions, insertions and deletions are types of SNP with the minor allele frequency being greater than or equal to one percent (Lander 1996; Vignal et al., 2002). SNP markers are one of the preferred genotyping approaches because they are abundant in the genome and for their use in automated analysis (Vignal et al., 2002).

Single Nucleotide Polymorphisms' are a gateway to the reasoning behind why certain animals are predisposed to perform in a variety of settings. The complexity of the issue lies' in the thought that there are breed specific SNP's which are more prevalent in individual breeds. SNP based research has the potential to provide information concerning animal population diversity and population evolution (origins, differentiation, and migrations) via SNP found significantly associated with different populations (Wanji et al., 2013). Thus development of an informative genetic system for beef cattle of breeding values and heterosis effects allows producers to determine the consequences of alternative selection and mating options (Garrick et al., 2009). With this information, livestock producers more rapidly improve livestock populations for economically important traits (Garrick et al., 2009).

SNP Associated with Growth Traits

Simultaneous improvement of antagonistically correlated traits poses a significant challenge for beef cattle breeders. The issue of reducing calf mortality by controlling birth weight while increasing subsequent growth has been well documented (Dickerson et al., 1974). Excessive calf birth weight was shown to be an important causative agent affecting the incidence and severity of dystocia (Bellows et al., 1971; Laster et al., 1973). Previous studies have reported that a molecular approach must be taken to address the issue of sacrificing birth weight for subsequent growth. More emphasis is placed on research correlated to subsequent growth after parturition. Sugimoto & associates (2012) identified a SNP associated with birth weight, A-326G. It was concluded cows with higher birth weights carried the A SNP substitution which correlated to the high rate of dystocia. It has been previously reported that an SNP in the Leptin gene is associated with increased milk production and tested for association with weaning weight in a herd of crossbred cows (Devuyst et al., 2013). Devuyst and associates (2013) reported cattle inheriting either CT or TT alleles for the SNP in the Leptin gene weaned significantly heavier calves than cows not inheriting one or both copies of the T allele. Single nucleotide polymorphisms are utilized also in post birth traits of economic importance such as weaning weight, and hip height.

SNP Associated with Carcass Quality Traits

Determining SNP's associated with carcass quality and composition is a vital goal in genomic research. Molecular marker information can be a useful tool for estimating breeding values and selecting animals for carcass traits. Additionally, selection processes could start earlier, even before phenotypic expression (Ferraz et

al., 2009). For example the Leptin gene located on Bos Taurus autosome 4 is considered to play a role in the regulation of appetite, energy partition, and body composition (Houseknecht et al., 1998; Baile et al., 2000). Two single nucleotide polymorphism; E2JW and E2FB were found to be associated with fat, lean yield, and grade fat while interacting on their effect on longissimus muscle tenderness (Schenkel et al., 2005). Remple & associates, (2012), reported five markers within DNA-Protein Kinase gene that were associated with fat thickness. Two of the markers rs41718998 and rs41718970 were associated with marbling score. Marker rs41719435 was associated with fat thickness, percent choice, yield grade, and retail product yield. Marker rs41624082 was associated with hot carcass weight. The final marker rs41726290 was associated with retail product yield fat yield. The Calpain1 (CAPN1) gene has been reported to harbor two known SNP that have an effect on meat tenderization; CAPN316, CAPN530 (Page et al., 2002, 2004).

A single nucleotide polymorphism was detected in the CAST gene characterized as a guanine to cytosine substitution that was reported to have decreased longissimus muscle tenderness across days of postmortem aging for animals expressing the cytosine nucleotide rather than guanine (Schenkel et al., 2006). Inheritance of the cytosine nucleotide is of importance to producers as well as there was a decrease in steaks rated unacceptable due to toughness for animals tested expressing the cytosine SNP (Schenkel et al., 2006).

Candidate Genes

A candidate gene is a gene of known physiological function used to predict their biological role in the variation observed in a particular trait (Karisa et al., 2013). Utilizing

the candidate gene approach can be extremely beneficial provided that the gene is associated with a true causative affect. It is best to utilize the candidate gene approach when testing for associations with complex traits where each gene has a relatively small effect on said trait (Karisa et al., 2013).

The Leptin Gene produces a hormone leptin that has been previously reported as a potential candidate gene (Stone et al., 1996). Leptin production has been considered to play a role in regulation of appetite, energy partition, and body composition (Houseknecht et al., 1998; Baile et al., 2000). Leptin is synthesized and expressed predominantly by adipocytes (Houseknecht et al., 1998) and relates to the feedback system that regulates long-term body fat weight and composition (Hossner, 1998). Schenkel and associates, (2005), reported 2 SNP in the Leptin gene that were associated with fat yield. UASMS3 and UASMS1 were found to be associated in a population of crossbred steers and heifers from the University of Geulph.

The Pit-1 gene has been examined as a genetic marker candidate gene (Zhao et al., 2004). The Pit-1 gene is a pituitary-specific transcription factor that is responsible for pituitary development in mammals (Cohen et al., 1997). In cattle, Pit-1 was found to be associated with body composition and milk yields (Renaville et al., 1997a). Zhao & associates utilized two polymorphisms from exon 3 of the Pit gene, Pit1I3H and Pit1I3NL. The two 2 SNP were genotyped in population of Angus cattle and utilized in an association study for growth and carcass trait. The results did not yield any genetic effects from Pit1I3H or PIT1I3L on growth or carcass traits.

IGF-1 Gene

The Insulin-like Growth Factor (IGF-1) is considered to be a regulatory factor that controls growth, differentiation, and the maintenance of various function in numerous tissue. Insulin Like Growth Factor that is circulating throughout the body is synthesized and secreted primarily by the liver (Bauman, 1992). However, several fetal and adult tissues also synthesize IGF-1. Growth hormone has been proven to be the major regulator of many processes throughout the body and acts by expressing many genes, one of which is IGF-1 (Sumantran et al., 1992; Ho and Hoffman, 1993; Lincoln et al., 1995).). The IGF-1 gene has been previously reported to be located on chromosome 5 (Grosse et al., 1999). Genes involved in the control of insulin-like growth factor I (IGFI) are primary candidates due to their influence on many traits related to growth rate and body composition (Hale et al., 2000; Thomas et al., 2007; Farber et al., 2006).

Thyroglobulin Gene

The Thyroglobulin gene (TG) is a precursor to various thyroid hormones with known endocrine roles in fat metabolism (Barendse, 1999). The thyroglobulin gene has been mapped to the centromeric region of chromosome 14 (Casas et al., 2005). A polymorphism in the 5' leader sequence of TG reported as TG5, has been previously reported to be associated with marbling and is the source of a commercially available DNA test (Barendse et al., 2004). The previously reported SNP TG5 is a C/T substitution in a repetitive element upstream from the promoter of the TG gene (Barendse et al., 1999). The TG5 gene marker is a tool of great importance in commercial application due to the fact that a small number of SNP-based markers that have been reported with associations to quantitative carcass or growth traits (Casas et

al., 2005). Eenennaam & associates, (2007), utilized the GeneSTAR Quality Grade marker panel that includes the TG5 gene marker on a population of charolais x angus cross and purebred Hereford animals. Eenennaam et al., 2007, did not report any significant association of the presence of the TG5 marker and marbling score. However, there was a trend reported toward increased quality grade in Charolais x Angus cross animals that were fed <250 days (Eenennaam et al., 2007). Furthermore, Casas & associates (2007), reported no significant associations with the TG SNP and marbling score for Bos Indicus animals.

There is a lack of research dealing with Bos Indicus cross animals and the TG SNP affecting marbling score, but the TG marker could be in linkage disequilibrium with functional alleles affecting fat deposition in Bos Indicus (Casas et al., 2007). More research is needed to validate previous findings and validate if the TG gene SNP is affecting fat deposition by its mode of action or by simple linkage.

Adiponectin Gene

Adiponectin (ADPOQ) was mapped near the QTL affecting marbling, ribeye muscle area, and fat thickness on BTA1. Shin and associates (2013) reported that Adiponectin modulates several biological processes including energy homeostasis, glucose, and lipid metabolism. It has been reported that Adiponectin is the most abundant protein secreted exclusively by the white adipose tissue in several mammalian species, and its' secretion is negatively correlated with adipose tissue mass (Kadowaki and Yamauchi, 2005). Significant associations were detected between the cluster of three ADPOQ loci separated by 166bp with carcass traits such as fat thickness and ribeye muscle area in Angus Cattle (Morsci et al., 2006). Previously

reported results indicate that ADPOQ is an excellent positional and functional candidate gene for further research when testing for associations with carcass quality and composition related traits.

Cast Gene

The calpain-calpastatin enzyme complex has been shown to regulate the rate of protein degradation. The complex works in such a way that calpain activity accelerates protein degradation while calpastatin activity inhibits this process, in both live and postmortem animals (Koochmaraie et al., 2002). The calpain-calpastatin system has also been previously reported by (Shackelford et al., 1995; Ferguson et al., 2001) to play a role in the ability of *Bos indicus* to thrive and perform in adverse conditions. As a result of this increased calpain-calpastatin activity previous reports have indicated *Bos indicus* cattle usually have tougher meat than purebred *Bos Taurus* cattle (Shackelford et al., 1995; Ferguson et al., 2001). The Calpastatin (CAST) gene, mapped to BTA 7 (Bishop et al., 1993), is considered a candidate gene for beef tenderness as consumers consider tenderness to be single most important trait in regards to meat quality (Miller et al., 1995). Shenkel et al., (2006) reported an SNP in the CAST gene, a G to C substitution, to have a significant effect on lowering the percentage of unacceptably tough steaks at 2 and 7 days postmortem on a population of crossbred steers and heifers.

MAS

Marker Assisted Selection (MAS) is the practice that strives to institute selection for animals that are pre-disposed to high performance in economically important traits with the use of single nucleotide polymorphisms, restriction fragment length

polymorphisms, and microsatellites. Rocha et al., 1992, proposed the idea that if mild to major genetic effects associated with a genetic marker could be identified and selected for, it could lead to a higher accuracy for selection. There are many traits in which direct selection is unattainable due to low heritability, cost of evaluation, occurrence later in life, or the traits are only measurable post mortem (David and Denise, 1998). The application of marker assisted selection by seed stock operators, would greatly improve selection for desirable traits and improve the quality of the overall end product (Macneil and Grosv, 2001). The practice would involve genotyping animals at a young age to see if the animal harbors the known marker. Producers would then be capable of knowing if the animal is predisposed to perform for an economically important trait and subsequently incorporate the animal into the breeding herd or cull them.

WGS

Whole Genome Selection is a form of marker-assisted selection in which genetic markers covering the whole genome are used as a selection tool (Goddard and Hayes, 2007). Most often genes affecting traits of economic importance are distributed throughout the genome and there are relatively few that have large effects with many more genes with progressively smaller effects (Thallman, 2009). It is a way to statistically analyze marker frequency between populations by looking at SNPs that are spread out across all chromosomes (BIF, 2010). Sellner & associates (2007), describes the statistical advantage of WGS as a sophisticated enhancement of single marker association mapping that allows for the entire genome to be analyzed simultaneously. The utilization of WGS has become feasible thanks to the large number of SNP

discovered by genome sequencing and new methods to efficiently genotype large number of SNP (Goddard and Hayes, 2007).

Bolormaa & associates (2011), conducted a study utilizing the whole genome selection approach with the purpose of finding more SNP associated with carcass quality to validate previous SNP results. The study utilized 54,000 SNP across the bovine genome to test for correlation with three economically important traits: intramuscular fat, meat tenderness, rump fat thickness (Bolormaa et al., 2011). The results reported indicated that validating prior findings may be a beneficial tool to progress MAS to the point of a common tool for producers to utilize (Bolormaa et al., 2011). The results from Bolormaa & associates (2011), revealed the importance of WGS to verify and discover new QTL or SNP that affect traits of economic importance.

CHAPTER III.

EVALUATION OF CALVES SIRED BY THREE PATERNAL SIRE BREEDS FOR GROWTH, PERFORMANCE, AND CARCASS TRAITS

Introduction

The cattle industry as a whole is a diverse market with a major emphasis on decreasing input cost and maximizing profit. Producers are faced with task of increasing productivity in traits of economic importance and increasing the accuracy of selection to remain profitable. Greiner (2009) reported that reproduction, growth, maternal ability, and carcass composition are traits that influence productivity and profitability of the beef enterprise (Greiner, 2009). Crossbreeding is a fundamental practice that has helped producers reach these goals (Greiner, 2009). The breeding of two separate breeds of cattle or crossbreeding offers two primary advantages, hybrid vigor and breed complementarities (Greiner, 2009). Hybrid vigor is when a crossbred offspring surpasses the production of their purebred parents in traits of economic importance and environmental adaptability (Greiner, 2009).

Coleman & associates (2012) reported that roughly 30% of the cow herd in the United States is located in the Gulf Coast region, but cattle in subtropical regions are exposed to problems associated with heat, parasite and disease exposure, and a seasonally impacted feed supply. Breed differences in performance characteristics are important genetic resources for improving the efficiency of beef production (Casas et al., 2011). As such, knowledge of breed composition can be a beneficial tool when selecting cattle for various areas of production (Kuehn et al., 2011).

The use of paternal sire breeds in a terminal based cow-herd is common practice for producers not retaining ownership of calves. Arango & associates (2002) reported that paternal breeds of cattle have been reported to sire calves with increased post weaning gains and frame size. Paternal breeds have also been reported to increase hot carcass weight and have variable quality grades which can influence pricing systems (Williams and Bennett, 1995). No one breed of cattle excels in every trait of economic importance. Diverse sire breed selection could potentially exploit heterosis and breed complementarity affects through crossbreeding to meet diverse market demands (Wheeler et al., 2011). The objective of the current study was to evaluate growth, performance and carcass quality and composition traits from a group of spring born calves sired by either Charolais, Braunvieh, or Simmental bulls.

Experimental Animals

The population of animals utilized in the current study was comprised of 110 crossbred calves born at the Louisiana State University Central Research Station crossbred unit in Baton Rouge, La. Calves in the current study were sired by Charolais, Simmental or Braunvieh bulls. The breed type of the dams has been previously described during the characterization of the Germplasm Evaluation VIII studies (Wheeler et al., 2011). All animals were handled while following the IACUC procedures put in place by the LSU AgCenter. Prior to breeding season the GPE VIII females were divided into three breeding groups ranging from 30-40 cows per group with two bulls per sired breed placed with them for the entire 75 day breeding season (April 15th – July 1st). The spring calving season started on January 15th, 2012 and ended April 1st, 2012.

Data was collected at Central Research Station throughout the calves' production cycle at the farm and consisted of sex, birth weight, weaning weight, and hip height. Ear notches were collected at birth when calf birth weight was collected and were subsequently used for DNA extraction. All calves were weighed within 24 hours of birth and color, sex, and sire breed was recorded. All bull calves were castrated at birth in conjunction with all previously described data collection procedures. The cow-calf pairs were then moved to rye grass pastures, and were maintained throughout spring. At approximately 205 days the calves from the spring calving group were weaned, and weaning weight and hip height measurements were recorded. Steers that met shipping standard criteria of weighing above 450 pounds and no visible ailments or injuries were vaccinated and shipped to Hitch Feedyards in Oklahoma for feedlot evaluation and subsequent carcass quality and composition measurements. The heifers were retained as potential replacements for incorporation into a three breed rotational crossbreeding that will be evaluated in future studies.

Statistical Analysis

The Mixed Model procedure of SAS (version 9.3, SAS Institute, Cary, NC) was utilized to test for significant differences in growth, performance, and carcass quality and composition traits between Charolais, Braunvieh, or Simmental calf groups. Birth weight (BW), weaning weight (WW), hip height (HH), ribeye area (REA), marbling score (MS), hot carcass weight (HCW), back fat thickness (BF), and yield grade (YG) were fit as random variables in the model. Breed and sex were fit into the model as fixed variables. The LSmeans function was utilized to evaluate any significantly different

means in evaluated performance and carcass traits among breeds. Statistical significance was assessed at $P < .05$.

Results

Individual sire breed groups and number of live calves born within sire breed groups by sex are illustrated in Table 2.0. There were a total of 44 Simmental sired calves born with 25 being steers and 19 heifers (Table 2.0). Twenty-five birth weight recordings were collected for Simmental steers and only twenty-two recordings for weaning weight and hip height. Nineteen birth weight, weaning weight, and hip height measurement were collected for Simmental sired heifers. There were a total of 32 Braunvieh sired calves born with 15 being steers and 17 heifers (Table 2.0). All calves, both steers and heifers lived through weaning and birth weight, weaning weight, and hip height measurements were collected accordingly. There were a total of 34 Charolais sired calves born with fifteen being steers and nineteen being heifers (Table 2.0). Fifteen birth weight, weaning weight, and hip height recording were collected for Charolais sired steers. Nineteen birth weight measurements were collected for Charolais sired heifers and eighteen weaning weight and hip height measurements were taken.

Table 2.1 Number of calves born per breed by sex

| Charolais | Braunvieh | Simmental | Total |
|------------------|------------------|------------------|--------------|
| Steer – 15 | Steer – 15 | Steer - 25 | Steer - 55 |
| Heifer – 19 | Heifer – 17 | Heifer - 19 | Heifer - 55 |

Simmental sired calves had a significantly ($P<.05$) heavier BW than calves sired by Charolais and Braunvieh bulls (Figure 2.3). When evaluating weaning weight no significant difference among breeds was detected (Figure 2.4). In regards to hip height, Braunvieh sired calves had a significantly ($P<.05$) shorter HH than calves sired by Simmental bulls. No significant differences were observed when comparing Charolais to Simmental and Braunvieh sired calves (Figure 2.5). No significant differences were detected when evaluating rib eye area, back fat thickness, marbling score, or yield grade among breeds (Figure 2.7, 2.8, 2.9, 2.10). Charolais sired calves had a significantly ($P<.05$) heavier HCW than Braunvieh sired calves. No significant differences were observed when comparing Simmental to Charolais and Braunvieh sired calves (Figure 2.6).

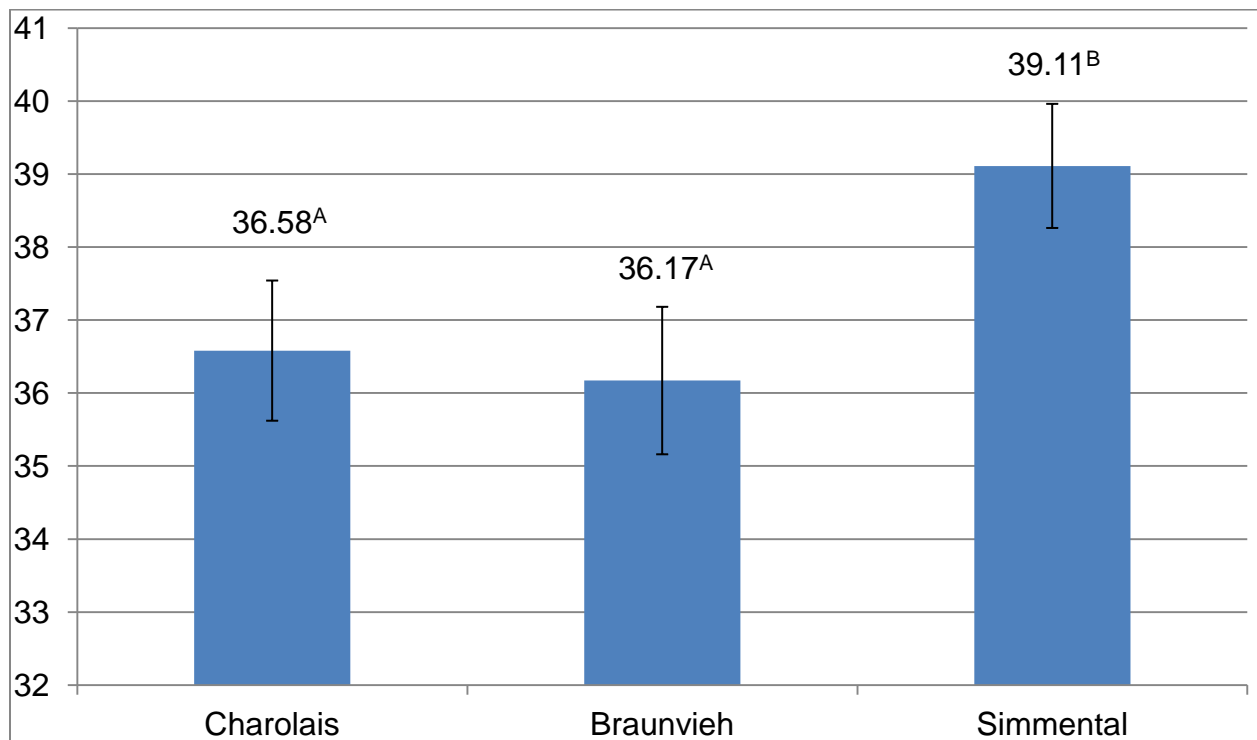


Figure 2.3 Average birth weight (kg) by sire breed

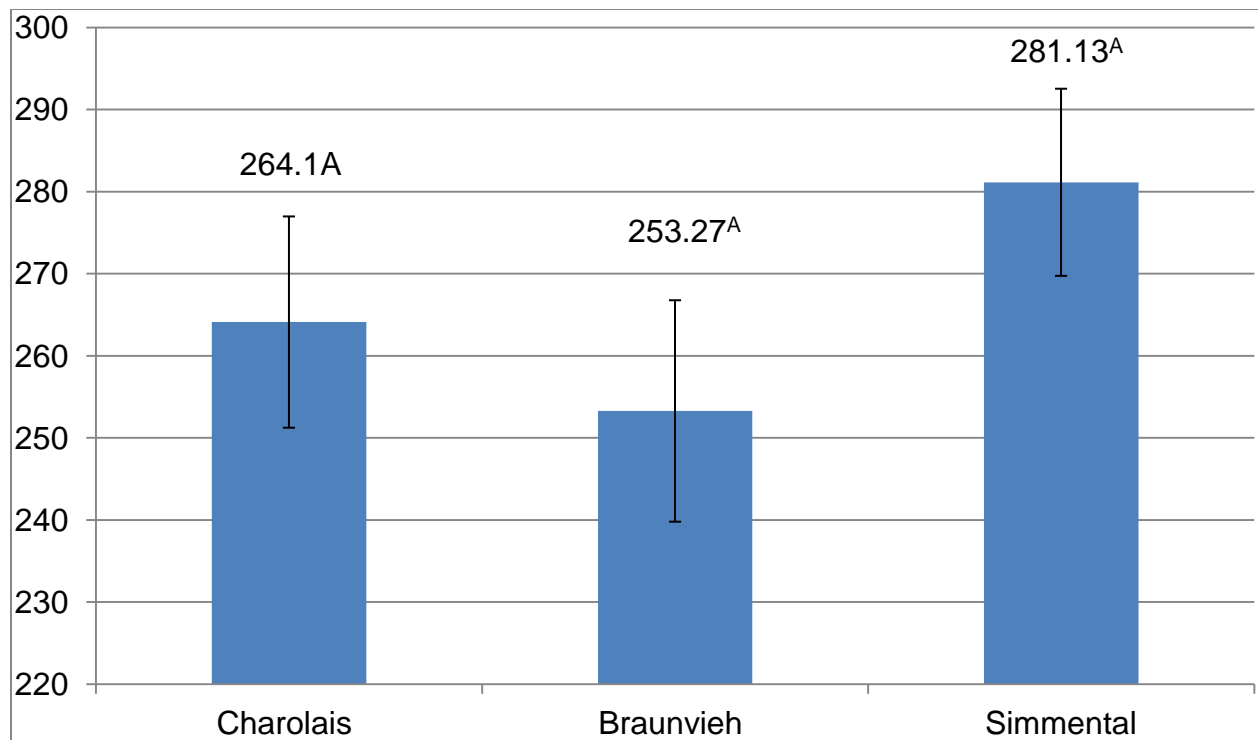


Figure 2.4 Average weaning weight (kg) by sire breeds

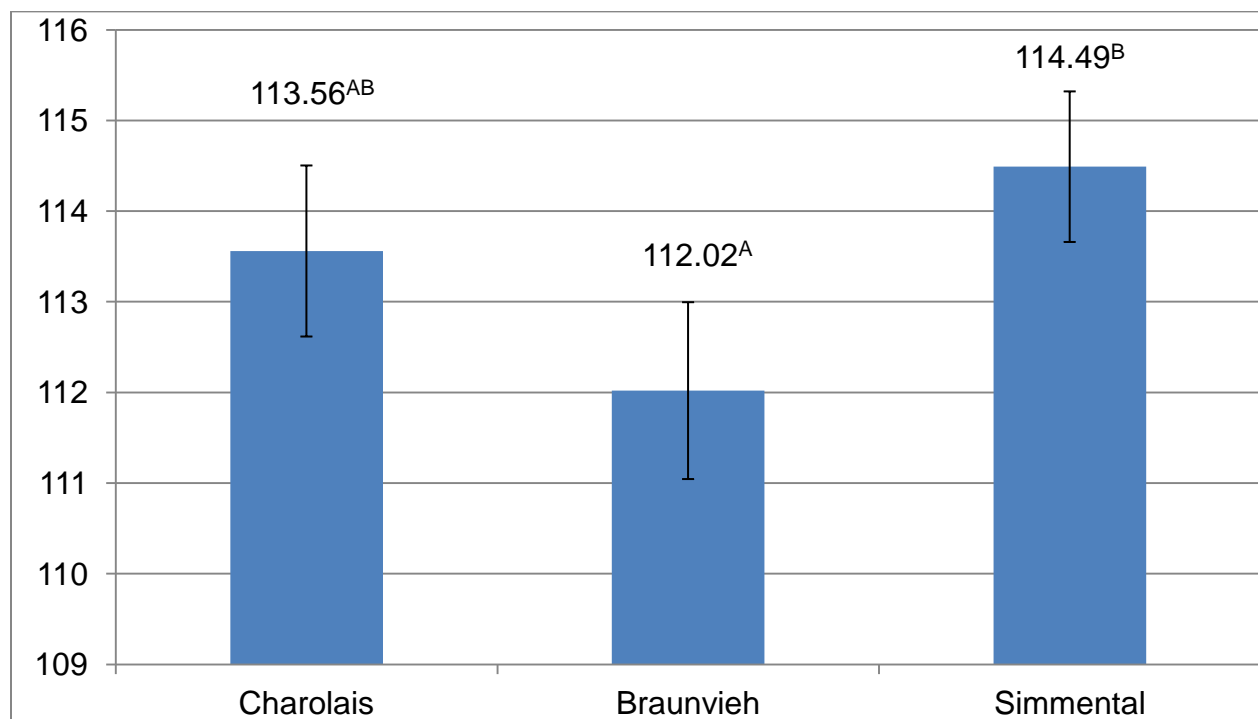


Figure 2.5 Average hip height (cm) by sire breed

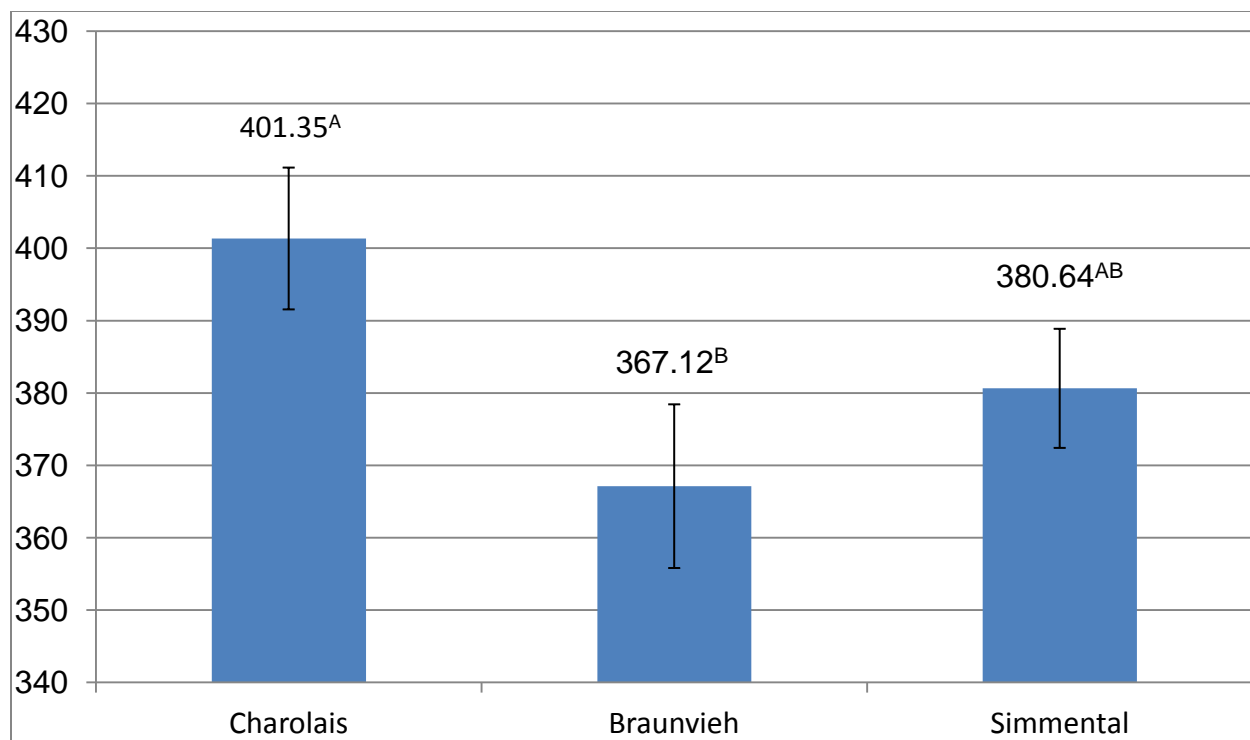


Figure 2.6 Average hot carcass weight (kg) by sire breed

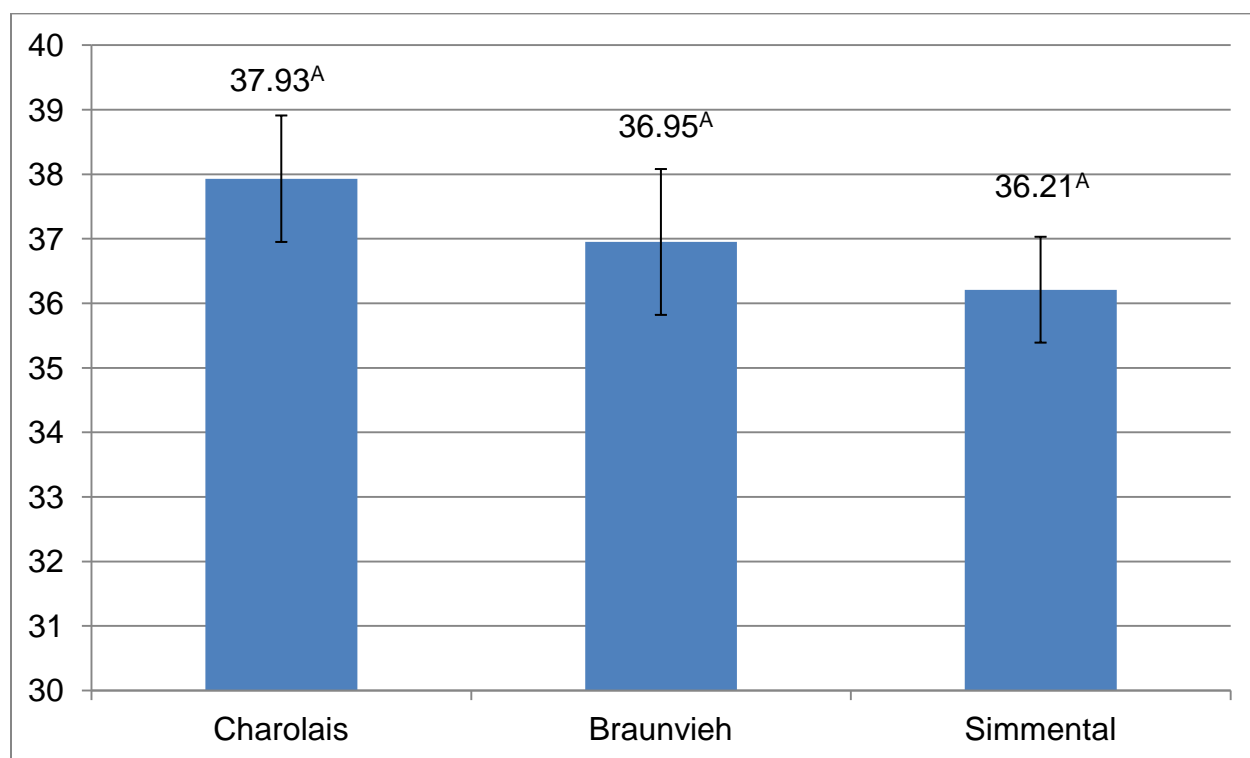


Figure 2.7 Average rib eye area (cm) by sire breed

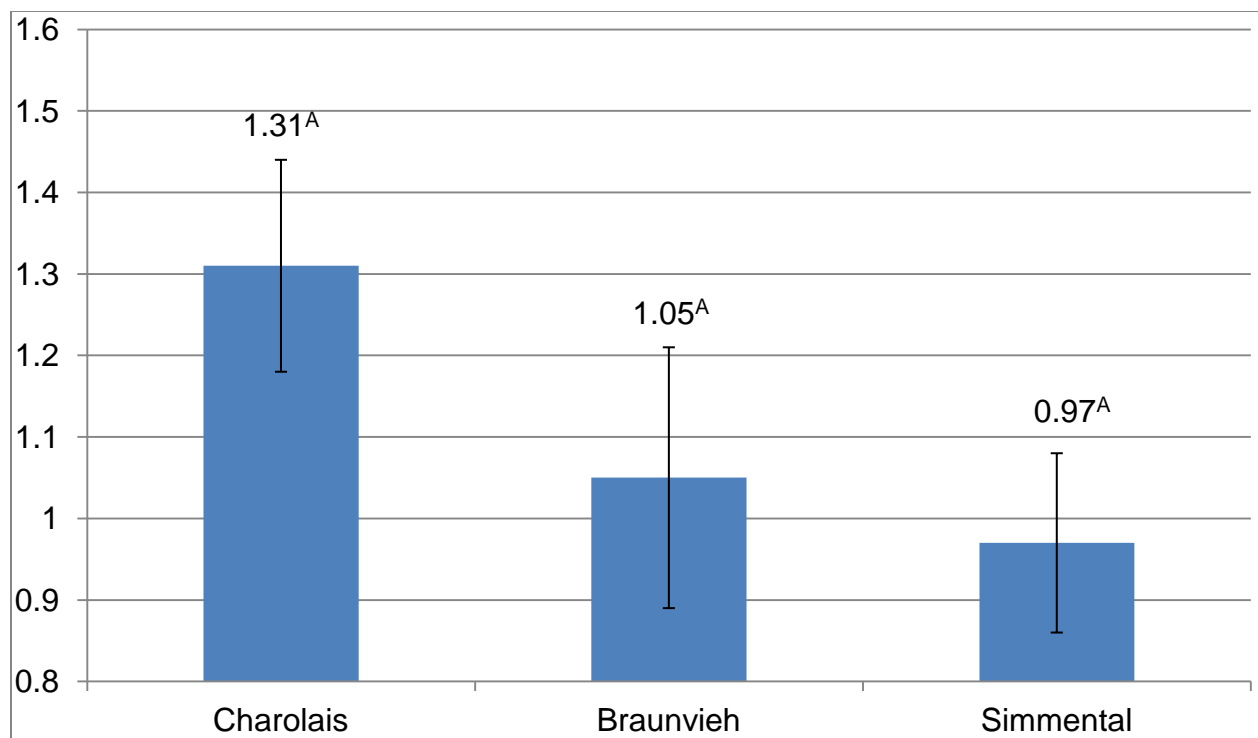


Figure 2.8 Average back fat thickness (cm) by sire breed

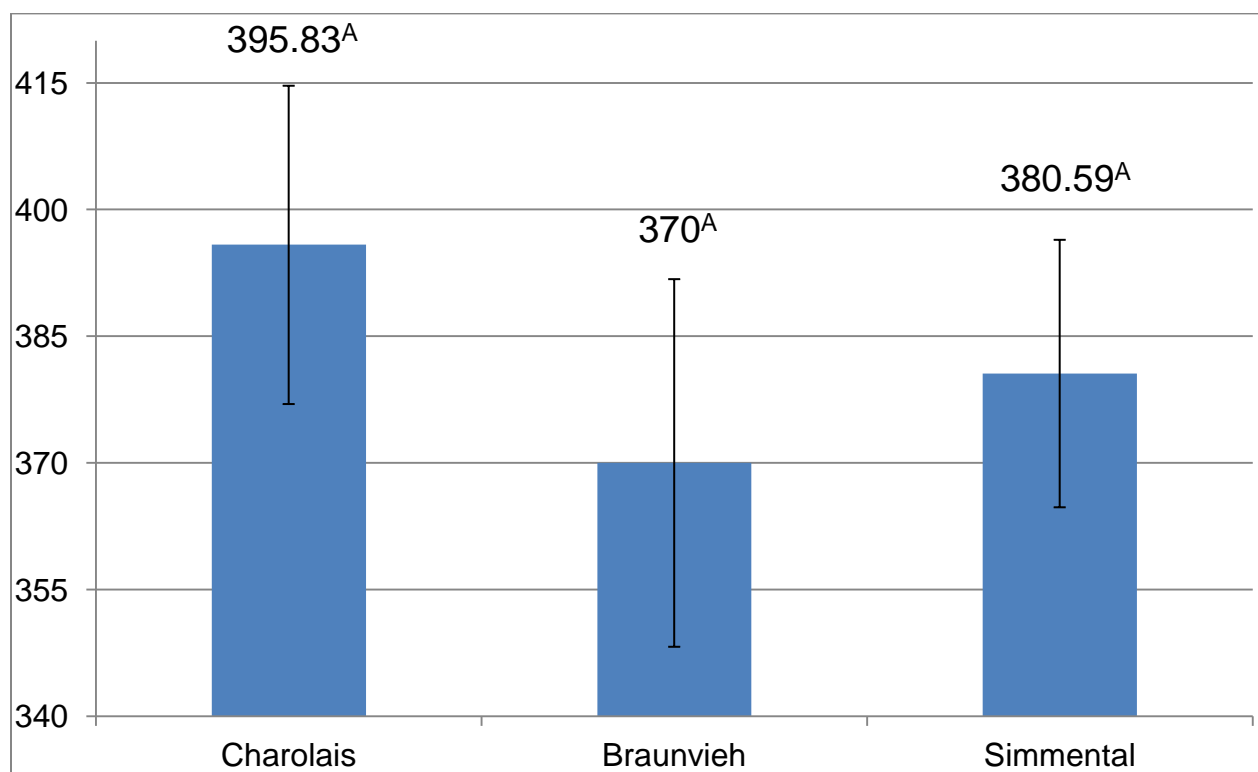


Figure 2.9 Average marbling score (units) by sire breed

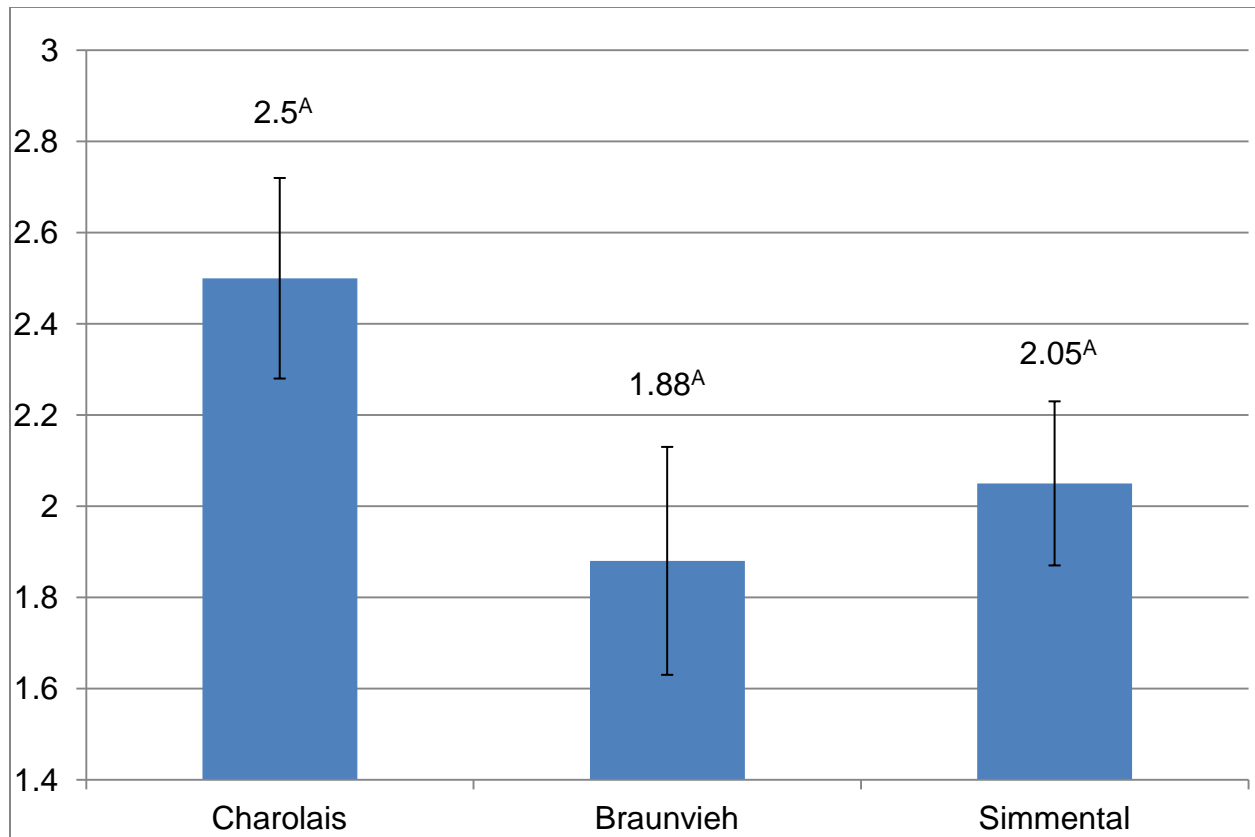


Figure 2.10 Average yield grade by sire breed

Discussion

Growth and performance traits that include birth weight, weaning, and hip height are of great importance to cattle producers as many terminal calves are sold at weaning. Birth weight is an economically important trait in beef cattle and is usually the first trait measured at birth (Utsunomiya et al., 2013). Furthermore, birth weight is related to growth traits, mature size, and carcass weight, along with a selection criterion for calving ease (Meyer et al., 1995; Bourbon et al., 1982). The majority of calves sold at auction are sold by the pound. For this reason, along with others, weaning weight has a direct effect on profit returned to the producer. Hip height is an indicator of frame size and future mature size of an animal.

The United States Department of Agriculture Meat Animal Research Center (MARC) in Clay Center, Nebraska has routinely conducted studies where the objective is to do comprehensive research on specific cattle breed performances. These studies evaluate several breeds and analyze cattle at various stages of the production scheme (Thallman et al., 1999). Research evaluating the performance of crossbred animals is important to producers as it provides alternative breeding animal selection. In the southeast region of the United States many producers utilize crossbred cattle herds with predominantly Angus or Charolais sires. This objective of the study herein was to evaluate the calf group performance for three paternal sire breeds (Charolais, Braunvieh, and Simmental) with growth, performance, and carcass quality and composition traits. Alternative sire breed selection could allow producers the knowledge to venture outside the normal sire breed selection and utilize bulls better suited for various production goals, or environments.

When evaluating birth weight, Simmental sired calves were shown to have the highest birth weight among sire breeds. Lawlor and associates (1984) reported similar results in which Simmental sired calves were significantly heavier at birth when compared to Hereford and Angus sired calves. Wheeler and associates (2007) reported that European breeds were leaner, heavier muscled, and had higher yielding carcasses compared to British breeds for a study conducted at the MARC center. The increased birth weight in the Simmental breed could possibly be a precursor for future increased carcass yield and larger mature size. This is in agreement with Arango & associates (2002) reports of British sired cattle were found to be lighter in weight and smaller in frame size when compared to continental or European sired calves. No significant

differences were observed for weaning weight among the sire breeds. When evaluating hip height at weaning, Braunvieh sired calves were significantly shorter than there Charolais and Simmental counterparts.

Carcass traits including hot carcass weight, ribeye area, backfat thickness, marbling score, and yield grade were chosen for breed by breed analysis. When evaluating hot carcass weight, Charolais sired calves yielded significantly larger carcasses. This is in agreement with Wheeler & associated (1996), where Charolais calves were reported to have the largest hot carcass weight compared to Gelbvieh, Pinzgauer, Shorthorn, Galloway, and Longhorn sired cattle. No significant differences among sire breeds were detected for ribeye area, backfat thickness, marbling score, or yield grade. Previous research by Wheeler & associates (1996), reported similar results in which Charolais sired calves were not significantly associated with increased rib eye area, increased back fat thickness, greater marbling score or quality yield grade when compared to other breeds previously mentioned. Limited breed comparisons have been conducted utilizing the Braunvieh breed of cattle. Knowledge of differences between breeds is necessary to evaluate the suitability of breeds for crossbreeding and to assess which of the breeds should be used as the sire breed for future offspring (Brandt et al., 2010). More research in the future with larger population sizes needs to be conducted to find potential significant differences among diverse sire breeds of cattle.

CHAPTER IV. EVALUATION OF SNP ON CANDIDATE GENES FOR GROWTH, PERFORMANCE, AND CARCASS TRAITS IN STEER CALVES SIRED BY BRAUNVIEH, CHAROLAIS, OR SIMMENTAL BULLS

Introduction

Identification of molecular markers associated with growth, performance, and carcass traits has become a major focus in the beef industry over the past few decades (Dekkers, 2004). The expression of growth and carcass related traits is often controlled by multiple genes rather than one, which compounds the difficulty in trying to understand inheritance and expression patterns (Zhao et al., 2004). Studies conducted in recent years have started utilizing the candidate gene approach. This approach utilizes genes of known physiological functions to evaluate their biological role in the variation observed in an economical important trait. Once significant associations are made with known candidate genes they can be further evaluated in different populations to validate the original findings. This is done in hopes of finding future mutations that producers can test for when trying to select breeding individuals to enhance the occurrence of a particular trait of interest.

Four previously reported candidate genes were chosen for analysis in this study including Insulin-like Growth Factor-1, Thyroglobulin, Adiponectin, and Calpastatin. Gene symbols were (IGF-1), (TG), (ADPOQ), and (CAST) respectively. IGF-1 was chosen for analysis because it has been previously reported to be associated with several major regulatory processes throughout the body such as growth, milk production, and aging (Sumantran et al., 1992; Ho and Hoffman, 1993; Lincoln et al., 1995). Grosse and associates (2009) reported the IGF-1 gene to be located on BTA 5

of the bovine genome. IGF-1 is stimulated by growth hormone and as a result is released from the liver (Bauman, 1992). TG has been mapped to centromeric region of chromosome 14 (Casas et al., 2005). Barendse and associates (1999) reported that TG is a precursor to various thyroid hormones with known endocrine roles in fat metabolism. Previous findings reported that a known polymorphism in the 5' region of TG known as TG5, has been significantly associated with marbling in certain studies (Barendse et al., 2004). ADPOQ, was chosen for analysis because it has been mapped near the QTL affecting marbling, ribeye muscle area, and fat thickness on BTA1 (Morsci et al., 2006). Significant associations were detected between the cluster of three ADPOQ loci separated by 166bp with carcass traits such as fat thickness and ribeye muscle area in Angus Cattle (Morsci et al., 2006). These finding suggest that ADPOQ is an excellent positional and functional candidate gene for further research when testing for associations with carcass quality and composition traits. The CAST gene was chosen because of the well known calpain-calpastatin enzyme complex that has been shown to regulate the rate of protein degradation. The complex works in such a way that calpain activity accelerates protein degradation while calpastatin activity inhibits this process, in both live and postmortem animals (Koohmaraie et al., 2002). The objective of this study was to evaluate potential SNP associations on four candidate genes with growth, performance, and carcass composition traits in a population of crossbred steers sired by Charolais, Braunvieh, and Simmental bulls.

Experimental Animals

The population of animals utilized in the current study was comprised of fifty-one steers calves born at the Louisiana State University Central Research Station crossbred unit in Baton Rouge, La. Calves in the current study were sired by Charolais, Braunvieh, or Simmental bulls. The breed type of the dams has been previously described during the characterization of the Germplasm Evaluation VIII studies (Wheeler et al., 2011). Prior to breeding season the GPE VIII females were divided into three breeding groups ranging from 30-40 cows per group with two bulls per sired breed placed with them for the entirety 75 day breeding season (April 15th – July 1st). The spring calving season started on January 15th, 2012 and ended April 1st, 2012.

Data was collected at Central Research Station throughout the calves' production cycle at the farm and consisted of sex, birth weight, weaning weight, and hip height. Ear notches were collected at birth when the calf was weighed and were subsequently used for DNA extraction. All calves were weighed within 24 hours of birth and color, sex, and sire breed was recorded. All bull calves were castrated at birth in conjunction with all previously described data collection procedures. The cow-calf pairs were then moved to rye grass pastures, and were maintained throughout spring. At approximately 205 days the calves from the spring calving group were weaned and weaning weight and hip height measurements were recorded. Steers that met shipping standard criteria of weighing above 450 pounds and having no visible ailments or injuries were vaccinated and shipped to Hitch Feed yards in Oklahoma for feedlot evaluation and subsequent carcass quality and composition measurements. Carcass quality and composition

measurements were recorded at nearby commercial packing plant.

Tissue Collection and DNA Extraction

Ear notch samples were collected for every calf currently being evaluated. Immediately after collection the notches were stored in a research freezer until further extraction procedures were performed. The extraction process is initiated by thawing frozen samples for placement into individually labeled 15ml tubes. The DNA from the ear notches was extracted using a saturated salt procedure previously described in Miller et al., 1988 (Appendix A). After DNA extraction, 51 two hundred microliter DNA working solutions were prepared with a combination of DNA and rehydration buffer. Unused buffy coat, extracted DNA, and working solutions were all stored at -4°C as a representative in the DNA bank or for future analysis.

SNP and Genotyping

Single Nucleotide Polymorphisms from previously reported results were chosen on candidate genes Insulin like Growth Factor, Thyroglobulin, Adiponectin, and Calpastatin. The SNP were selected from the dbSNP website (<http://www.ncbi.nlm.nih.gov/projects/SNP/>). Single nucleotide polymorphisms were selected at equal distributions across each candidate gene so that SNP coverage was achieved across the entire gene. Single nucleotide polymorphisms, allele substitutions, and forward and reverse primer sequences are reported in the tables 3.1, 3.2, 3.3, 3.4, and 3.5. Single nucleotide polymorphism genotyping was performed by NEOGENE, Inc. (Lincoln, Nebraska) via the Sequonome procedure utilizing Iplex technology.

Table 3.1 Single nucleotide polymorphisms ID, allele substitution, and forward and reverse primer sequences utilized for amplification and visualization of genotypes for TG

| SNP ID | Allele Substitution | Forward Sequence | Reverse Sequence |
|-------------|---------------------|-----------------------------|-----------------------------|
| rs110501231 | C/T | ATACGATTGGGATGTTTCTGTACCAA | TTTGGGTGGAAGGGGAGAATGAGTC |
| rs135059985 | C/T | GGGTCTTGAGCACTGGAACCTTCATTT | TCTAAGATGGCAGTCCTGCACTTGG |
| rs136379742 | C/T | CCCAGGCCCCAGGGTCTTCTCGCT | CCATCAGAACAGGAGCCCTTGGCAA |
| rs378567477 | C/T | ACATGAGGAGCTCCTGTCAAGCTGA | GCCCCAGCATCACCTCCCCAGGAA |
| rs383724494 | C/T | ATCTGTCAAAGATTATAAGAAATATG | ACATCCAGACCACAGAGGCGGCATG |
| rs136849694 | A/G | TGCTGGCCACAGCGGTGGGGAGGCG | GGCCCCCACCAGGAAGTGGACGGTG |
| rs379996188 | C/T | ACTGACC CAGAAGGCCT TCCTGGGT | CACAAAGGTACAGGTGAGGGGGCCAC |
| rs110999400 | C/G | GGGCTATATTCCACGTCTAATATCA | GAAAGACGGAAGAGTGAGGGGCGATGG |
| rs133473042 | C/G | CTCCTTCTGTGACTCACTTCACTCAG | CAATCTCTGGGCCCATCCATGTTGCT |
| rs110191002 | C/T | ACTTTTAATCTTCTCTCCATTTGCTGC | TGGGTTCTTGTTGTTATCCATTCTA |
| rs109182502 | C/T | CTGGATGTTTCTGTCCAGCCATTGCT | GGCTCTGTTTCAGGGACCGGCTGATT |
| rs382252585 | A/G | GAAAAGTAATTTCCAAATTACAACCT | AGTGCCTAGGTACCTGGATGTTTCTG |
| rs380627374 | A/G | CAGTTCATACACACACGCAGTAGGGG | TCGCCTTTGAAAAGTAATTTCCAAAT |
| rs132813094 | A/C | TTTAAAAGGTAATACCCTGACTCCTG | GGCAACGGCCGCCTTCTGATTTCAA |
| rs133980693 | A/G | CTCCCTTCTCCTGACATCTCTGGCAA | CCTTCCCACCTGGCTCGGTTACAAAT |
| rs377997897 | C/G | AAACTTAAATACAATTTTCTGAGTCA | CAATGACTGTGAGATATTGTTAGCCT |
| rs109830314 | C/T | CACGTCTCCTGCACTGCAGGTGGATT | ACCACTGAACTAGCAGGGAAACCCAA |
| rs29021775 | A/G | ATCAGTGGCTATGCCTGCTTTCCTCT | AGAGTCAGGTTATATTTTAGTAAGGA |
| rs109188488 | C/G | GGGTGGGTTCTGGTTCTGGTCAGTCT | TGGCTTTTCCATGCCACAGACTTAAT |
| rs110946911 | G/T | TTCACAGAGCTTTGTCTTTCACGAGC | TAGGCTGTAATTCATCCCTGCAGTTG |
| rs110553649 | A/C | GACACGAGTCAGCAACTCACATACA | TTGTTAGTGTCTTTGAATATCAGAC |
| rs110187386 | C/G | ATAACTCTGCAAACTAAAGCAAGAGA | TTCAAAAAAAAAAATCTTCTATACGT |
| rs378900777 | C/T | GGCTGGGTTTATTGTTACTGTTTGGT | CCAACCTTTTTGTGATGCCATAGACT |
| rs381723399 | C/T | ATTTTCTTCCCAACCCAGGGATCAAA | ATCTCCTGCATTGCAGGCAGATTCTT |
| rs384062524 | C/G | TGGCTGCGTGGGATGAAAGGAGAGG | TCTGAAGCAGGGATTACCACAGAGGC |
| rs110616947 | A/C | GGAGAATTAATAATCCATCCATTGA | CCATCCATTCACTTACACAGTTGCTG |
| rs109068240 | C/T | CTTCTCCAACACTGCAGCTCAAAAGC | TTCTTCAACTCAGCCTTCTTTATGGT |
| rs379467464 | C/T | TCAAGTGAATGGCTGGATGGCTGACT | CTGCGTGGGATGAACAGGAGAGGTC |
| rs386026054 | C/G | ATAACTCTGCAAACTAAAGCAAGAGA | TTCAAAAAAAAAAATCTTCTATACGT |
| rs134743669 | A/G | CCTGGTAGACTACAACCGAGGGGGT | GAGTCAGACACGAGTCAGCAACTCAC |

Table 3.2 Single nucleotide polymorphisms ID, allele substitution, and forward and reverse primer sequences utilized for amplification and visualization of genotypes for ADPOQ

| SNP ID | Allele Substitution | Forward Sequence | Reverse Sequence |
|-------------|---------------------|-----------------------------|-----------------------------|
| rs380209068 | A/G | ATCAAAACACCCTAACAATTCCCAAA | GATTACTAAATCCAAAACCTCTA |
| rs209727017 | C/T | CCAACCAATTTGAAACACTCTACTGA | ATACTTTGTTGTTAAGAAAGGGAGCA |
| rs380391978 | A/C | TGGGAAATAATGCAAATAGAAGGAAA | TTTCCCAAATTTTATTTGGGGCAGAA |
| rs208856619 | C/T | CCCTGGTCTCTCCTGGTTGCCAGCTC | TTAGGATGGGGGTACCCAGGAAATAG |
| rs383535987 | A/G | TACATTGCTCCTCTGTGCCTGTGCAC | TCCTTCTCTTTCCTCTCCACTTGGAG |
| rs133746968 | A/G | CCTAGCTTCATGCACACTGGCTGTGG | AAAGGAGGCCATGTCTGTCAAGCACC |
| rs385926794 | C/T | GTAGGAAGGAGTGAAAGGATCAGGGA | CCAGGAAGGGGCTGGAGAATTCCAGA |
| rs385383133 | A/G | TCCCCCTCCCTTTAGGGAAGGAATCT | ACCTTTCAAGGGTTTCTGAATGCAGG |
| rs210865525 | C/T | CCAATGTTGTTAATGCAGCAATGGAC | CTGCTTAGAAAAACCCCAATCTGTTG |
| rs208093103 | A/G | GGGCCGGTACAAGAGACACAGGAGAC | TTTGATGCCTGGGTCAGGAAGATCCC |
| rs378178622 | C/T | ATTCAGTAGCCTGGGTGACCTGGAAG | TGTGTATAAGCCCCTGGAAAAGAGTA |
| rs209050698 | A/G | AACTTCCAAGTACACAACACGTATTA | TAGAGGCCCATGACCCAGTTTTGATC |
| rs382192949 | A/G | AACTGATGCTCAGCTTTAGAGTACT | AGCAACTCATGTTTTAAAAAATAAGG |
| rs208699764 | C/G | ATCAAATACATGTGAGCCTTTCCTCC | GTGTCATGGTTACCATTCAGTCCCCT |
| rs211230641 | A/T | GACTTTAGTCCCCAAATCGCATGGCT | GCTTACCTGGCTGTCAGACAGGAACA |
| rs378724414 | A/T | TACGCCAATATTTTATTTTATTGTG | TCTGATTTATTTTGGGTTTGTATTAC |
| rs382701614 | C/T | GAAGCGACTTAGCAGCAGCAGCAGCA | TAGTAAAGTAATGCTCAAAATTCTCC |
| rs386011953 | A/G | GCTGTGAAAGTGCTGCACTCAATATG | AAATTTGGAAAACCTCAGCAGTGTCCA |
| rs379059851 | C/T | AAGCATGTGAATGCAGAGTTCCAAAG | CAAGAAGAGATAAGAAAGCCTTCCTC |
| rs381854487 | A/G | AAAGGCTTTGGCGTAGTCAATAAAGC | ATGCCTATCAGAGCCACACAAGTCA |
| rs383391069 | C/T | TCTTTTAATTTTCATGGCTGCAGTCAC | GCAGTGATTTTGGAGCCCAGAAAAAT |
| rs380790166 | C/T | TCCTTTCAGTCCAAGGGACTCTCAAG | TCTACCTCACCACAGTTCAAAAGCAT |
| rs382644882 | C/G | TGGCTTTGCCAAGACAGAGTTCAAGGT | ACACTGCCACTTTAAATCTAAGCCTT |
| rs381911082 | G/T | TCAGGTGGTACAAGTGGTATAGAACC | ATGCCAATACAGAAGACACAAGAGAC |
| rs209420330 | C/T | ACAGGGAACCTGCAGTATAGCAAGAAA | TACATCCTTTCCTCTCTGGCCTGAAC |
| rs210607551 | C/T | GCCCCTGGCAGCTCCTTTGGCTTGCC | AGGGAACCTGGTGCAACCCAGTTCGG |
| rs384076273 | C/T | CCTAGAACTTGAATCAGTCGTCCTTA | AGGTGTGGAATCAGAGCCACAGACTT |
| rs209743114 | C/G | ACCTATTAATAGACTATTTACAATA | GTGAGAACAGGTTCACTTAAAGTATC |
| rs210601794 | C/T | TCAGTGCAGAGATGCTGAACTTTCAA | TTTAAACTGTGTGTGCTCCCTATTT |
| rs210258853 | A/G | AAAAGGAAATACAGAGAGAGGAGGGA | GAAGGAAAAGAGAAAGGAAGAGGGAA |

Table 3.3: Single nucleotide polymorphisms ID, allele substitution, and forward and reverse primer sequences utilized for amplification and visualization of genotypes for IGF-1

| SNP ID | Allele Substitution | Forward Sequence | Reverse Sequence |
|-------------|---------------------|----------------------------|----------------------------|
| rs137605212 | A/G | CCACTCCCCTGGCAAGGACCCAGGAG | AAGATGACCCTCCTTCTGCTTTTTTC |
| rs137250028 | C/T | GGACAGAGCACATGACTAGCCAATGA | GCTATAATGGAATTGATTAGTTAGT |
| rs136493168 | A/G | AACCACTTCCTGCTCCAAGTACAGGA | AAAGCAACAACCTTATGGCTAGCTAG |
| rs135968955 | G/T | AGATAAAGGAGTCTAAAATGTTCTTT | GTCACTATTTGAATCCAAGATTCTC |
| rs135711837 | G/T | GCGTACTTTTGATGGATTAAATATTA | AAAATATTAAGGAAATTCAAATCTA |
| rs135230510 | A/G | TGAAACACTAGGCTCGCATTAAGGTG | GGAATCTCGGAGGCTGAGGACGGCT |
| rs134494935 | C/T | TTCCATCTTTGATTCTGTGTTAAGAA | CCCAGCCACTAAGCACCCCATTCTA |
| rs133980322 | G/T | GCATTATTACTGTATCCATTTACAGA | GAGGAAATGGAGATTTAGCAAGGGT |
| rs133253110 | C/T | GGCTTAGAGAATTCCATGGACCATAC | CATGGGGTTGCAAAGAGTCGGACAT |
| rs132951819 | G/T | CTTTGCAATAATATATTACCAACAAT | TCCCTTTGTTGAATGCTTTCTATTA |
| rs132665612 | A/G | CAGTGAGTCAAGTGGACTGGAATAAA | TAGGGGAGAATTATTCCTGTCTGAG |
| rs110959643 | A/G | TCCCACACAAGATGGAGAGCAGACCC | TCCCAGTATTTGGGGAGGCCCATCA |
| rs110266103 | A/G | AGCAGTGAAACAATGCAAAGGTGATC | TTAAGTTTTTCCACATTGCTACTTG |
| rs109327701 | A/G | AAGAATCGCAGTGTACTGGGTGAGAT | TGAACACCCAGCCATGCCTTAACT |
| rs109227434 | C/T | TCCATTTYCCTTTGGCCTGTCAAGCC | GTAGTRGTTGTGTGTACCCATAAGA |
| rs109199979 | C/T | CAGCCTTTCTAGGACCTCAGCTAGAC | ACAGGTGAAAGAAGAAAAATCTGAA |
| rs109074329 | C/T | TAAGAGGAAGAAAGGRGGAGCATACC | GCCCAGCTAGCCCTGTTGACCAACT |
| rs109022910 | A/G | TGCGAGCCTAGTGTTTCAGCGGGGCC | TGGCACGTTTTGCAGATTTTGGATG |
| rs43434843 | A/T | AAACAATAAAGAACTTGCTTAGGAAT | AAAAAGTTTGAAATGAGTGGCCCCA |
| rs43434842 | A/G | ATATGTGGGGGGCATATGTAAACTCA | ATGCCTATCAGAGCCACACAAGTCA |

Table 3.4: Single nucleotide polymorphisms ID, allele substitution, and forward and reverse primer sequences utilized for amplification and visualization of genotypes for CAST

| SNP ID | Allele Substitution | Forward Sequence | Reverse Sequence |
|-------------|---------------------|------------------------------|-----------------------------|
| rs137780582 | G/T | ATAAGAAAATAAAAAAAAAAAAAAGAAC | CTTAACCCCACGATCAGAAAATAAC |
| rs137777861 | G/T | CTGGTGAATGAATAAACTAATATATG | TGAATTGAGCCATCACGTAATACTC |
| rs137726884 | A/G | AAACTTACCATTTAAATGTTCCCTG | AAGTTGCAAGTCTTTGATAGACTCC |
| rs137722600 | C/T | CCAAGCAGAAGACGTGGGTCTATCT | GGGGTTGGAAAGATCCCCTGGAGAA |
| rs137711215 | A/G | TAAAATGTTAGAGAAAAGCAAAGGGA | TTCAGGGAAACATGAGGATTTTCA |
| rs137662301 | C/T | CATGGGGTCAACAAAGAGTCAGACATG | CTCAGCAGTCAGACAAACAGCAAGG |
| rs137601357 | C/T | CAGAACTCAGGCTGGTGAAAAAGCCC | GGTCCCCAAGGTCAGTCATTTCTG |
| rs137561617 | A/T | ATTGAATTTAACTTTTACATGCTGAT | TTCAGTATCTAAAGGATATTTATTG |
| rs137374423 | C/G | TCATTTTCCTTTCTGTTCTCAGACT | TATAATTTTCAGTTGTCCTATTTTTG |
| rs137330201 | A/G | CTCATCTGCTCACCTTTATCATTTT | TTGATTCTTTGCTAGCAGTATTGGC |
| rs137265200 | C/T | ACAAAGAGTCAGACATGTCTCAGCAG | CAGACAAACAGCAAGGGTGTTAATG |
| rs137211570 | A/C | CCAGGCCTCCCTGTCCATCACCAACT | CCGGAGTTTACTCAAACATCATGTCC |
| rs137151719 | G/T | AGTTCAAGTGTAAGTGTATTCTTCCA | AAGGAAAAGCATTTCTTATCTCTC |
| rs137140434 | G/T | TTCAGTTATTATATGTCTCCACTCTA | AATTTTTTTTTGGTTTCTTTTAGA |
| rs137104571 | C/G | AGTGGTTCTGCTTCTGGGCCAAAGAG | GCTGAAAAGTGAATTCTCTCAGTCG |
| rs136982429 | C/T | CCAGGCAAGAATACTGGAGTGGGTG | CATTTCTCCTCCAGGAGATCTTCC |
| rs136939207 | G/T | TAAACATTCATTATTACCTATATTGT | TTTTGCTTTTTGAAGTCAGAATACC |
| rs136882857 | C/T | CAGATCTCCTGCCTGGGAAGGGCCTT | ATTCATTTCAATTCATTCAAACCTCTT |
| rs136875549 | C/T | ATAACTTCCACCTTTTGTGGCTTTTT | CCTAAGCGTTTGGGGTGCTCCTGTG |
| rs136873074 | C/T | CTCCCGAACTACAGGCGGATTCTTTA | GAACTGAGCTAGGAGGGAAGCCCAG |

Statistical Analysis

The Mixed Model procedure of SAS (version 9.3, SAS Institute, Cary, NC) was utilized to evaluate potential SNP association on four candidate genes with growth, performance, and carcass quality and composition traits for animals inheriting different SNP genotypes. Birth weight (BW), weaning weight (WW), hip height (HH), ribeye area (REA), marbling score (MS), hot carcass weight (HCW), back fat thickness (BF), and yield grade (YG) were fit as random variables in the model. Breed and SNP genotype were fit into the model as fixed variables. The analyses evaluated traits previously mentioned specifically for significant differences among individual animals for SNP chosen for each candidate gene. The LSMeans function of SAS was utilized to identify significant differences ($P < .05$) when evaluating mean performance of traits among different individual SNP genotypes. Statistical significance was assessed at $P < .05$ and a statistical trend at $P < .10$. Due to the small sample size a statistical trend ($P < .10$) was utilized to alleviate any possible Type II statistical errors made and to identify markers for further investigation with a larger sample size

Results

Five SNP (rs379467464, rs380209068, rs210865525, rs208093103, and rs109327701) were significantly associated ($P < .05$) birth weight and located on the TG, ADPOQ, and IGF-1 gene (Table 3.5). Four additional SNP (rs135059985, rs380391975, rs385926794, and rs109227434) were included in Table 3.5 for possible future analysis in a larger population size. Animals inheriting the homozygous TT genotype for marker rs379467464 displayed a larger birth weight than those inheriting the heterozygous CT genotype (Table 3.6). Animals inheriting the homozygous TT genotype for marker

rs380209068 displayed a larger birth weight than those inheriting the heterozygous AT genotype (Table 3.6). For marker rs210865525, animals inheriting the homozygous AA genotype displayed a larger birth weight than those inheriting the heterozygous GA genotype (Table 3.6). Animals inheriting the homozygous CC genotype for marker rs208093103 displayed a larger birth weight than those inheriting the heterozygous TC genotype (Table 3.6). For marker rs109327701, animals inheriting the homozygous AA genotype displayed a heavier birth weight than those inheriting the homozygous GG genotype (Table 3.6).

Table 3.5 Level of significance, number of animals from each genotype, and standard error for SNP significantly associated with Birth Weight (kg)

| Trait | Gene | SNP ID | Allele | Minor Gen. Frequency | Het Gen. Frequency | Major Gen. Frequency | P-value |
|-------|-------|-------------|--------|----------------------|--------------------|----------------------|----------|
| BWT | TG | rs135059985 | T/C | 3 | 17 | 15 | 0.0796** |
| BWT | TG | rs379467464 | C/T | 0 | 39 | 12 | 0.0273* |
| BWT | ADP | rs380209068 | A/T | 0 | 1 | 39 | 0.0133* |
| BWT | ADP | rs380391975 | G/A | 0 | 7 | 30 | 0.0794** |
| BWT | ADP | rs385926794 | G/T | 2 | 15 | 27 | 0.0053* |
| BWT | ADP | rs210865525 | G/A | 0 | 3 | 38 | 0.0174* |
| BWT | ADP | rs208093103 | T/C | 0 | 4 | 39 | 0.0415* |
| BWT | IGF-1 | rs109227434 | C/T | 3 | 22 | 26 | 0.0793** |
| BWT | IGF-1 | rs109327701 | G/A | 4 | 29 | 18 | 0.0499* |

[†]BWT = Birth Weight

Representation of the minor allele is located on the left

Number of animals inheriting each genotype

* Superscripts differ P<.05 indicate significance

** Superscripts differ P<.10 indicate statistical trend

Eight SNP (rs110501231, rs136849694, rs378900777, rs381723399, rs380209068, rs136939207, rs137140434, and rs137726884) were significantly associated (P<.05) with weaning weight and located on the TG, ADPOQ, and CAST genes (Table 3.7). Four other SNP (rs110999400, rs109830314, rs384062524, and

rs109327701) were included in Table 3.7 for future consideration in studies with a larger sample size. Animals inheriting the homozygous TT genotype for marker rs110501231

Table 3.6 Single nucleotide polymorphisms significantly associated with Birth Weight (kg) and least square means comparisons between reported genotypes

| Trait | Gene | SNP | Allele | Minor Gen. Mean | Het Gen. Mean | Major Gen. Mean |
|-------|-------|-------------|--------|--------------------------|--------------------------|-------------------------|
| BWT | TG | rs135059985 | T/C | 34 ± 3.17 ^{ab} | 33.3 ± 1.3 ^b | 37.7 ± 1.4 ^a |
| BWT | TG | rs379467464 | C/T | 0 | 35.6 ± .82 ^b | 39.5 ± 1.5 ^a |
| BWT | ADP | rs380209068 | A/T | 0 | 48.9 ± 5.2 ^b | 35.5 ± .81 ^a |
| BWT | ADP | rs380391975 | G/A | 0 | 32.2 ± 2.1 ^b | 36.3 ± 1.0 ^a |
| BWT | ADP | rs385926794 | G/T | 31.03 ± 3.4 ^a | 39.3 ± 1.2 ^b | 34.4 ± .94 ^a |
| BWT | ADP | rs210865525 | G/A | 0 | 42.9 ± 3.0 ^b | 35.2 ± .83 ^a |
| BWT | ADP | rs208093103 | T/C | 0 | 41.1 ± 2.6 ^b | 35.4 ± .83 ^a |
| BWT | IGF-1 | rs109227434 | C/T | 29.6 ± 3 ^a | 36.8 ± 1.1 ^b | 36.9 ± 1.0 ^b |
| BWT | IGF-1 | rs109327701 | G/A | 32.4 ± 2.6 ^b | 35.6 ± 1.0 ^{ab} | 38.6 ± 1.2 ^a |

¹BWT = Birth Weight

Representation of the minor allele is located on the left

* Superscripts differ P<.05 indicate significance

** Superscripts differ P < 0.10 indicate statistical trend

^{a,b} Superscripts indicate difference within row

displayed a larger weaning weight than those inheriting the heterozygous TC and homozygous CC genotype (Table 3.8). Animals inheriting the homozygous AA genotype for marker rs136849694 displayed a larger weaning weight than those inheriting the heterozygous AC and homozygous CC genotype (Table 3.8). For marker rs378900777, animals inheriting the homozygous TT genotype displayed a larger weaning weight than those inheriting the heterozygous TC and homozygous CC genotype (Table 3.8).

Animals inheriting the homozygous AA genotype for marker rs381723399 displayed a larger weaning weight than those inheriting the heterozygous GA and homozygous GG genotype (Table 3.8).

Table 3.7 Level of significance, number of animals from each genotype, and standard error for SNP significantly associated with Weaning Weight (kg)

| Trait | Gene | SNP ID | Allele | Minor Gen. Frequency | Het Gen. Frequency | Major Gen. Frequency | P-value |
|-------|-------|-------------|--------|----------------------|--------------------|----------------------|----------|
| WW | TG | rs110501231 | T/C | 6 | 23 | 9 | 0.0355* |
| WW | TG | rs136849694 | A/C | 1 | 12 | 22 | 0.0030* |
| WW | TG | rs110999400 | A/C | 8 | 21 | 9 | 0.0838** |
| WW | TG | rs109830314 | C/G | 0 | 12 | 25 | 0.0608** |
| WW | TG | rs378900777 | T/C | 7 | 22 | 11 | 0.0420* |
| WW | TG | rs381723399 | G/A | 8 | 20 | 9 | 0.0460* |
| WW | TG | rs384062524 | T/C | 0 | 32 | 32 | 0.0992** |
| WW | ADP | rs380209068 | A/T | 0 | 1 | 39 | 0.0023* |
| WW | IGF-1 | rs109327701 | G/A | 4 | 29 | 18 | 0.0668** |
| WW | CAST | rs136939207 | T/C | 1 | 44 | 4 | 0.0076* |
| WW | CAST | rs137140434 | G/T | 1 | 5 | 7 | 0.0303* |
| WW | CAST | rs137726884 | A/G | 3 | 25 | 21 | 0.0024* |

¹WW = Weaning Weight

Representation of the minor allele is located on the left

Number of animals inheriting each genotype

*Superscripts differ P<.05 indicate significance

**Superscripts differ P<.10 indicate statistical trend

For marker rs380209068, animals inheriting the homozygous TT genotype displayed a heavier weaning weight than those inheriting the heterozygous A/T genotype (Table 3.8). For marker rs136939207, animals inheriting the homozygous CC genotype displayed a larger weaning weight than those inheriting the heterozygous TC and homozygous CC genotype (Table 3.8). Animals inheriting the homozygous G/G genotype for marker rs137140434 displayed a heavier weaning weight than those inheriting the heterozygous GT and homozygous TT genotype (Table 3.8). Animals inheriting the homozygous AA genotype for marker rs137726884 displayed a larger weaning weight than those inheriting the heterozygous AG and homozygous GG genotype (Table 3.8).

Table 3.8 Single nucleotide polymorphisms significantly associated with Weaning Weight (kg) and least square means comparisons between reported genotypes

| Trait | Gene | SNP | Allele | Minor Gen. Mean | Het Gen. Mean | Major Gen. Mean |
|-------|-------|-------------|--------|------------------------|------------------------|------------------------|
| WW | TG | rs110501231 | T/C | 253 ± 16 ^b | 300 ± 8.5 ^a | 298 ± 13 ^a |
| WW | TG | rs136849694 | A/C | 161 ± 36 ^a | 290 ± 10 ^b | 301 ± 8.1 ^b |
| WW | TG | rs110999400 | G/C | 261 ± 15 ^a | 301 ± 8.9 ^b | 296 ± 13 ^{ab} |
| WW | TG | rs109830314 | C/G | 0 | 310 ± 12 ^b | 282 ± 8.4 ^a |
| WW | TG | rs378900777 | T/C | 259 ± 15 ^b | 296 ± 8.6 ^a | 306 ± 12 ^a |
| WW | TG | rs381723399 | G/A | 306 ± 15 ^b | 300 ± 9.3 ^b | 262 ± 13 ^a |
| WW | TG | rs384062524 | T/C | 0 | 315 ± 14 ^a | 287 ± 7.1 ^a |
| WW | ADP | rs380209068 | A/T | 0 | 413 ± 37 ^b | 290 ± 6.0 ^a |
| WW | IGF-1 | rs109327701 | G/A | 303 ± 18 ^{ab} | 302 ± 7.0 ^b | 276 ± 9.0 ^a |
| WW | CAST | rs136939207 | T/C | 334 ± 36 ^b | 297 ± 5.5 ^b | 236 ± 18 ^a |
| WW | CAST | rs137140434 | G/T | 156 ± 49 ^a | 322 ± 27 ^b | 304 ± 19 ^b |
| WW | CAST | rs137726884 | A/G | 223 ± 20 ^b | 292 ± 7.0 ^a | 304 ± 7.1 ^a |

¹WW = Weaning Weight

Representation of the minor allele is located on the left

*Superscripts differ P<.05 indicate significance

**Superscripts differ P < 0.10 indicate statistical trend

^{a,b}Superscripts indicate difference within row

When evaluating SNP across 4 candidate genes for hip height no significance (P<0.5) was detected for any SNP (Table 3.9). However, three SNP (rs133473042,

Table 3.9 Level of significance, number of animals from each genotype, and standard error for SNP significantly associated with Hip Height (cm)

| Trait | Gene | SNP ID | Allele | Minor Gen. Frequency | Het Gen. Frequency | Major Gen. Frequency | P-value |
|-------|-------|-------------|--------|----------------------|--------------------|----------------------|----------|
| HH | TG | rs133473042 | A/C | 0 | 14 | 34 | 0.0823** |
| HH | ADP | rs210607551 | A/G | 5 | 30 | 10 | 0.0775** |
| HH | IGF-1 | rs109327701 | G/A | 4 | 29 | 18 | 0.0668** |

¹HH = Hip Height

Representation of the minor allele is located on the left

Number of animals inheriting each genotype

*Superscripts differ P<.05 indicate significance

**Superscripts differ P<.10 indicate statistical trend

rs210607551, and rs109327701) were observed trending (P<.10) and located on TG, ADPOQ, and IGF-1 gene. The SNP were included in Table 3.10 for consideration in future analysis with a larger sample size.

Table 3.10 Single nucleotide polymorphisms significantly associated with Hip Height (cm) and least square means comparisons between reported genotypes

| Trait | Gene | SNP | Allele | Minor Genotype Mean | Het Genotype Mean | Major Genotype Mean |
|-------|-------|-------------|--------|-------------------------|------------------------|-------------------------|
| HH | TG | rs133473042 | A/C | 0 | 105 ± 4.5 ^b | 115 ± 2.8 ^a |
| HH | ADP | rs210607551 | A/G | 96.0 ± 7.4 ^a | 114 ± 3.1 ^b | 114 ± 5.3 ^{ab} |
| HH | IGF-1 | rs109327701 | G/A | 304 ± 18 ^{ab} | 302 ± 7.0 ^b | 277 ± 8.6 ^a |

¹HH = Hip Height

Representation of the minor allele is located on the left

*Superscripts differ P<.05 indicate significance

**Superscripts differ P < 0.10 indicate statistical trend

^{a,b}Superscripts indicate difference within row

Two SNP (rs378567477 and rs29021775) were significantly associated (P<.05) with hot carcass weight and were located on the TG gene (Table 3.11). Animals inheriting the heterozygous TC and homozygous CC genotype for marker rs378567477 displayed a larger hot carcass weight than those inheriting the homozygous CC genotype (Table 3.12). For marker rs29021775, animals inheriting the homozygous CC

Table 3.11 Level of significance, number of animals from each genotype, and standard error for SNP significantly associated with Hot Carcass Weight (kg)

| Trait | Gene | SNP ID | Allele | Minor Gen. Frequency | Het Gen. Frequency | Major Gen. Frequency | P-value |
|-------|------|-------------|--------|----------------------|--------------------|----------------------|---------|
| HCW | TG | rs378567477 | T/C | 5 | 18 | 22 | 0.0046* |
| HCW | TG | rs29021775 | T/C | 13 | 7 | 23 | 0.0486* |

¹HCW = Hot Carcass Weight

Representation of the minor allele is located on the left

Number of animals inheriting each genotype

*Superscripts differ P<.05 indicate significance

**Superscripts differ P<.10 indicate statistical trend

genotype displayed a larger hot carcass weight than those inheriting the heterozygous TC genotype (Table 3.12).

Table 3.12 Single nucleotide polymorphisms significantly associated with Hot Carcass Weight (kg) and least square means comparisons between reported genotypes

| Trait | Gene | SNP | Allele | Minor Gen. Mean | Het Gen. Mean | Major Gen. Mean |
|-------|------|-------------|--------|------------------------|------------------------|------------------------|
| HCW | TG | rs378567477 | T/C | 326 ± 8.3 ^b | 380 ± 8.3 ^a | 392 ± 6.5 ^a |
| HCW | TG | rs29021775 | T/C | 377 ± 11 ^{ab} | 354 ± 14 ^b | 392 ± 6.7 ^a |

¹HCW = Hot Carcass Weight

Representation of the minor allele is located on the left

* Superscripts differ P<.05 indicate significance

** Superscripts differ P < 0.10 indicate statistical trend

^{a,b} Superscripts indicate difference within row

When evaluating SNP across 4 candidate genes for rib eye area no significance (P<.05) was detected for any SNP (Table 3.13). One SNP (rs135059985) was observed trending (P<.10) and located on the TG gene. It was included in Table 3.14 for possible future analysis in a larger population size.

Table 3.13 Level of significance, number of animals from each genotype, and standard error for SNP significantly associated with Ribeye Area (cm)

| Trait | Gene | SNP ID | Allele | Minor Gen. Frequency | Het Gen. Frequency | Major Gen. Frequency | P-value |
|-------|------|-------------|--------|----------------------|--------------------|----------------------|----------|
| REA | TG | rs135059985 | T/C | 3 | 17 | 15 | 0.0692** |

¹REA = Rib eye area

Representation of the minor allele is located on the left

Number of animals inheriting each genotype

* Superscripts differ P<.05 indicate significance

** Superscripts differ P<.10 indicate statistical trend

Table 3.14 Single nucleotide polymorphisms significantly associated with Ribeye Area (cm) and least square means comparisons between reported genotypes

| Trait | Gene | SNP | Allele | Minor Gen. Mean | Het Gen. Mean | Major Gen. Mean |
|-------|------|-------------|--------|--------------------------|------------------------|-------------------------|
| REA | TG | rs135059985 | T/C | 39.0 ± 1.9 ^{ab} | 36.0 ± .8 ^b | 38.3 ± 0.9 ^a |

¹REA = Rib eye area

Representation of the minor allele is located on the left

* Superscripts differ P<.05 indicate significance

** Superscripts differ P < 0.10 indicate statistical trend

^{a,b} Superscripts indicate difference within row

Six SNP (rs136849694, rs385383133, rs137140434, rs137374423, rs137662301, and rs137726884) were significantly associated ($P < .05$) with back fat thickness and located on TG, ADPOQ, and CAST genes (Table 3.15). Four SNP

Table 3.15 Level of significance, number of animals from each genotype, and standard error for SNP significantly associated with Back Fat (cm)

| Trait | Gene | SNP ID | Allele | Minor Gen. Frequency | Het Gen. Frequency | Major Gen. Frequency | P-value |
|-------|------|-------------|--------|----------------------|--------------------|----------------------|----------|
| BF | TG | rs378567477 | T/C | 5 | 18 | 22 | 0.0567** |
| BF | TG | rs136849694 | A/C | 1 | 12 | 22 | 0.0011* |
| BF | TG | rs29021775 | T/C | 13 | 7 | 23 | 0.0630** |
| BF | ADP | rs385383133 | C/G | 0 | 5 | 15 | 0.0354* |
| BF | ADP | rs21060755 | A/G | 5 | 30 | 10 | 0.0523** |
| BF | CAST | rs137140434 | G/T | 1 | 5 | 7 | 0.0178* |
| BF | CAST | rs137374423 | C/G | 3 | 16 | 20 | 0.0230* |
| BF | CAST | rs137601357 | C/T | 9 | 30 | 10 | 0.0534** |
| BF | CAST | rs137662301 | C/T | 0 | 7 | 40 | 0.0149* |
| BF | CAST | rs137726884 | A/G | 3 | 25 | 21 | 0.0265* |

[†]BF = Back Fat

Representation of the minor allele is located on the left

Number of animals inheriting each genotype

*Superscripts differ $P < .05$ indicate significance

**Superscripts differ $P < .10$ indicate statistical trend

(rs378567477, rs29021775, rs21060755, and rs137601357) were observed trending ($P < .10$) and included in included Table 3.15 for use in future analysis with a larger population size. Animals inheriting the homozygous AA genotype for marker rs136849694 displayed greater back fat thickness than those inheriting the heterozygous AC and homozygous CC genotype (Table 3.16). For marker rs385383133, animals inheriting the homozygous GG genotype displayed greater back fat thickness than those inheriting the heterozygous CG genotype (Table 3.16). Animals inheriting the homozygous GG genotype for marker rs137140434 displayed greater back fat thickness than those inheriting the heterozygous GT genotype (Table 3.16).

Animals inheriting the CC genotype for marker rs137374423 displayed greater back fat thickness than those inheriting the heterozygous CG and homozygous GG genotype (Table 3.16). Animals inheriting the heterozygous CT genotype for marker rs137662301 displayed greater back fat thickness than those inheriting the homozygous TT genotype (Table 3.16). For marker rs137726884, animals inheriting the AA genotype for marker rs137726884 displayed greater back fat thickness than those inheriting the heterozygous AG and homozygous GG genotype (Table 3.16).

Table 3.16 Single nucleotide polymorphisms significantly associated with Back Fat (cm) and least square means comparisons between reported genotypes

| Trait | Gene | SNP | Allele | Minor Gen. Mean | Het Gen. Mean | Major Gen. Mean |
|-------|------|-------------|--------|--------------------------|--------------------------|--------------------------|
| BF | TG | rs378567477 | T/C | 0.52 ± .27 ^a | 1.06 ± .12 ^{ab} | 1.20 ± .09 ^b |
| BF | TG | rs136849694 | A/C | 2.67 ± .41 ^a | 0.94 ± .11 ^b | 1.17 ± .08 ^b |
| BF | TG | rs29021775 | T/C | 1.07 ± .15 ^{ab} | 0.69 ± .19 ^b | 1.22 ± .09 ^a |
| BF | ADP | rs385383133 | C/G | 0 | 0.67 ± .24 ^b | 1.30 ± .13 ^a |
| BF | ADP | rs210607551 | A/G | 1.30 ± .21 ^{ab} | 1.00 ± .08 ^a | 1.48 ± .18 ^b |
| BF | CAST | rs137140434 | G/T | 2.57 ± .47 ^a | 0.86 ± .20 ^b | 1.45 ± .16 ^{ab} |
| BF | CAST | rs137374423 | C/G | 2.01 ± .33 ^a | 0.98 ± .11 ^b | 1.11 ± .01 ^b |
| BF | CAST | rs137601357 | C/T | 1.50 ± .17 ^a | 1.05 ± .09 ^b | 0.93 ± .18 ^b |
| BF | CAST | rs137662301 | C/T | 0 | 1.60 ± .20 ^a | 1.03 ± .08 ^b |
| BF | CAST | rs137726884 | A/G | 1.97 ± .31 ^a | 0.98 ± .11 ^b | 1.12 ± .11 ^b |

¹BF = Back Fat

Representation of the minor allele is located on the left

* Superscripts differ P<.05 indicate significance

** Superscripts differ P < 0.10 indicate statistical trend

^{a,b} Superscripts indicate difference within row

Two SNP (rs29021775 and rs110946911) were significantly associated (P<.05) with marbling score and located on the TG gene (Table 3.17). Two additional SNP (rs210865525 and rs137104571) were observed trending (P<.10) and included in Table 3.17 for possible future analysis in a larger population size. Animals inheriting the homozygous CC genotype for marker rs29021775 displayed a greater marbling score than those inheriting the heterozygous TC genotype (Table 3.18). For marker

rs110946911, animals inheriting the homozygous CC genotype displayed a greater marbling score than those inheriting the heterozygous TC genotype (Table 3.18).

Table 3.17 Level of significance, number of animals from each genotype, and standard error for SNP significantly associated with Marbling Score (units)

| Trait | Gene | SNP ID | Allele | Minor Gen. Frequency | Het Gen. Frequency | Major Gen. Frequency | P-value |
|-------|------|-------------|--------|----------------------|--------------------|----------------------|----------|
| MS | TG | rs29021775 | T/C | 13 | 7 | 23 | 0.0470* |
| MS | TG | rs110946911 | T/C | 2 | 23 | 23 | 0.0480* |
| MS | ADP | rs210865525 | G/A | 0 | 3 | 38 | 0.0565** |
| MS | CAST | rs137104571 | G/C | 7 | 22 | 16 | 0.0556** |

¹MS = Marbling Score

Representation of the minor allele is located on the left

Number of animals inheriting each genotype

*Superscripts differ P<.05 indicate significance

**Superscripts differ P<.10 indicate statistical trend

Table 3.18 Single nucleotide polymorphisms significantly associated with Marbling Score (units) and least square means comparisons between reported genotypes

| Trait | Gene | SNP | Allele | Minor Gen. Mean | Het Gen. Mean | Major Gen. Mean |
|-------|------|-------------|--------|------------------------|-----------------------|-----------------------|
| MS | TG | rs29021775 | T/C | 378 ± 20 ^{ab} | 322 ± 26 ^b | 397 ± 13 ^a |
| MS | TG | rs110946911 | T/C | 387 ± 44 ^{ab} | 408 ± 15 ^b | 356 ± 14 ^a |
| MS | ADP | rs210865525 | G/A | 0 | 453 ± 37 ^b | 377 ± 11 ^a |
| MS | CAST | rs137104571 | G/C | 383 ± 28 ^{ab} | 407 ± 15 ^b | 344 ± 18 ^a |

¹MS = Marbling Score

Representation of the minor allele is located on the left

*Superscripts differ P<.05 indicate significance

**Superscripts differ P < 0.10 indicate statistical trend

^{a,b}Superscripts indicate difference within row

Three SNP (rs110616947, rs380209068, and rs137601357) were significantly associated (P<.05) with yield grade and located on the TG, IGF-1, and CAST genes (Table 3.19). Four SNP (rs110553649, rs134743669, rs137374423, and rs137662301) were observed trending (P<.10) and were included in Table 3.19 for possible future analysis in a larger population size. Animals inheriting the homozygous GG genotype

Table 3.19 Level of significance, number of animals from each genotype, and standard error for SNP significantly associated with Yield Grade

| Trait | Gene | SNP ID | Allele | Minor Gen. Frequency | Het Gen. Frequency | Major Gen. Frequency | P-value |
|-------|-------|-------------|--------|----------------------|--------------------|----------------------|----------|
| YG | TG | rs110553649 | C/T | 3 | 27 | 21 | 0.0723** |
| YG | TG | rs110616947 | A/G | 10 | 26 | 12 | 0.0381* |
| YG | TG | rs134743669 | C/G | 9 | 18 | 15 | 0.0791** |
| YG | IGF-1 | rs380209068 | A/T | 0 | 1 | 39 | 0.0305* |
| YG | CAST | rs137374423 | C/G | 3 | 16 | 20 | 0.0927** |
| YG | CAST | rs137601357 | C/T | 9 | 30 | 10 | 0.0437* |
| YG | CAST | rs137662301 | C/T | 0 | 7 | 40 | 0.0849** |

¹YG = Yield Grade

Representation of the minor allele is located on the left

Number of animals inheriting each genotype

*Superscripts differ P<.05 indicate significance

**Superscripts differ P<.10 indicate statistical trend

for marker rs110616947 displayed a greater yield grade than those inheriting the

homozygous AA genotype (Table 3.20). Animals inheriting the homozygous TT

Table 3.20 Single nucleotide polymorphisms significantly associated with Yield Grade and least square means comparisons between reported genotypes

| Trait | Gene | SNP | Allele | Minor Gen. Mean | Het Gen. Mean | Major Gen. Mean |
|-------|-------|-------------|--------|--------------------------|--------------------------|--------------------------|
| YG | TG | rs110553649 | C/T | 2.34 ± .43 ^{ab} | 1.80 ± .17 ^b | 2.42 ± .17 ^a |
| YG | TG | rs110616947 | A/G | 1.80 ± .30 ^b | 2.10 ± .15 ^{ab} | 2.70 ± .25 ^a |
| YG | TG | rs134743669 | C/G | 2.70 ± .30 ^a | 1.91 ± .18 ^b | 2.13 ± .19 ^{ab} |
| YG | IGF-1 | rs380209068 | A/T | 0 | 0.55 ± 0.7 ^b | 2.20 ± .12 ^a |
| YG | CAST | rs137374423 | C/G | 3.30 ± .60 ^a | 2.01 ± .18 ^b | 2.14 ± .16 ^b |
| YG | CAST | rs137601357 | C/T | 2.50 ± .30 ^b | 2.30 ± .14 ^b | 1.50 ± .28 ^a |
| YG | CAST | rs137662301 | C/T | 0 | 2.70 ± .33 ^a | 2.10 ± .13 ^a |

¹YG = Yield Grade

Representation of the minor allele is located on the left

*Superscripts differ P<.05 indicate significance

**Superscripts differ P < 0.10 indicate statistical trend

^{a,b}Superscripts indicate difference within row

genotype for marker rs380209068 displayed a greater yield grade than those inheriting

the heterozygous CT genotype (Table 3.20). For marker rs137601357, animals

inheriting the homozygous TT genotype displayed a greater yield grade than those inheriting the heterozygous CT and homozygous TT genotype (Table 3.20).

Discussion

Thryoglobulin (TG) marker (rs379467464, rs110501231, rs136849694, rs378900777, rs381723399, rs378567477, rs29021775, rs136849694, rs29021775, rs1109469911, and rs110616947) were significantly associated ($P < .05$) with growth, performance, and carcass quality and composition traits including birth weight, weaning weight, hot carcass weight, back fat thickness, marbling score, and yield grade. Additional markers for TG were observed trending and included in without being statistically significant. These marker need to be evaluated in studies with a larger population size to determine possible significant associations with traits of economic importance. TG markers (rs29021775 and rs11094691) are of particular interest due to their significant association with marbling score as these results contradict previous results by Casas & associates (2005), where no significant associations were found with TG gene markers and marbling score.

Several Adiponectin markers (rs379467464, rs380209068, rs210865525, rs208093103, rs380209068, and rs385392133) were found to be significantly associated with growth, performance, and carcass quality and composition traits that included birth weight, weaning weight, and back fat thickness. Multiple ADPOQ markers were observed trending on the threshold of significance and were included as they should be considered in populations with a much larger sample size. Several ADPOQ markers were observed trending. Morsci and associates (2006) reported significant associations with a cluster of three ADPOQ loci with carcass traits such as fat thickness

and ribeye muscle area in Angus Cattle. These findings are in agreement with backfat thickness measurement in the study herein as marker rs385393133 was significantly associated with back fat thickness. Further research in populations of purebred *Bos taurus* and crossbred *Bos indicus* herds needs to be performed to further validate these results.

Insulin like Growth Factor 1 markers (rs109327701 and rs380209068) were found to significantly associated with growth, performance, and carcass quality and composition traits including birth weight and yield grade. Markers that were observed trending were included as they could be utilized in study populations with larger sample sizes. Efforts to validate previous findings by Machado (2003) and Andrade (2008) where IGF-1 mutations were reported to be associated with growth and production traits were of minimal success as only two markers were found to be associated milk birth weight and yield grade. Further research in populations of terminal sire crossbred calves needs to be performed to increase the number of markers significantly associated with these traits of interest.

CAST markers (rs136939207, rs137140434, rs137726884, rs137140434, rs137374423, rs137662301, rs137726884, and rs137601357) were found to be significantly associated with growth, performance, and carcass quality and composition traits that included weaning weight, back fat thickness, and yield grade. As previously mentioned markers that were observed trending and on the threshold of being significant were included as they are candidates for further research in studies with a larger population size. The calpain-calpastatin system has also been previously reported by (Shackelford et al., 1995; Ferguson et al., 2001) to play a role in the ability

of *Bos indicus* to thrive and perform in adverse conditions. As a result of this increased calpain-calpastatin activity *Bos indicus* cattle have tougher meat than purebred *Bos Taurus* cattle (Shackelford et al., 1995; Ferguson et al., 2001). The previously mentioned results are in agreement with finding in the study herein as the only traits with significant associations were back fat thickness and yield grade. The possibility of increased calpastatin in *Bos indicus* animals having a result on carcass traits such as marbling could have an effect on why no significance was observed in this population of animals. Markers rs137140434, rs137374423, rs137662301, rs137726884 were significantly associated with back fat thickness which is importance and should be validated in future studies with a larger sample size. Research performed in populations of crossbred animals is vital as traits of economic importance have a direct effect on the profitability of producers. Increasing the accuracy of genomic selection through research will enable producers to select animals' harboring these known mutations. Several markers across candidate genes TG, ADPOQ, IGF-1, and CAST were found to be significantly associated a variety of traits of economic importance. More analysis containing these marker and markers observed trending needs to be performed to validate these findings before their incorporation into marker assisted selections programs.

CHAPTER V. SUMMARY

The studies presented herein represent the variation in economically important traits from offspring sired by three paternal breeds of cattle. Growth and carcass related traits are of great value and importance to producers as they dictate the return on investment or profit a producer receives. A vast majority of cattle producers sell calves at weaning while some retain ownership. In these scenarios producers would be interested in breed performance for traits such as birth weight, weaning weight, and hip height. Arango & associates (2002) reported that paternal breeds of cattle in previous research sired calves with increased post weaning gains and frame size which. Knowledge of post weaning growth trends would be of value for producers retaining ownership of their cattle through the feedlot.

The objective of the first study was to evaluate growth, performance and carcass quality and composition traits. After interpretation of data, Simmental sired calves were significantly ($P<.05$) associated with an increased birth weight value compared to Braunvieh and Simmental sired calves. It is important to note that no single breed was significantly associated with increased weaning weight values. Braunvieh sired calves were significantly associated with a shorter hip height value at weaning compared to Simmental calves. In regards to carcass traits, Charolais sired calves were significantly associated with a heavier hot carcass weight compared to Braunvieh sired calves. However no significant difference was observed between Charolais and Simmental sired calves for hot carcass weight. Overall Simmental sired calves were heavier at birth and Braunvieh sired calves were significantly shorter at weaning compared to Simmental calves. Simmental bulls could be of potential value to producers throughout

the southeastern United States when considering pre weaning growth as a trait of importance to their individual operation. It is important to note that there was limited performance testing utilizing the Braunvieh breed. Research comparing popular breeds of cattle as well as alternative breeds provides producers with valuable knowledge needed to select the proper bull to achieve performance goals.

The second study in the research herein utilized the candidate approach to test SNP within in gene for association with growth and carcass related traits such as birth weight, weaning weight, hip height, hot carcass weight, marbling score, rib eye area, back fat thickness, and yield grade. Identification of molecular markers associated with growth, performance, and carcass traits has become a major focus in the beef industry over the past few decades (Dekkers, 2004). Within the current study 13 SNP were found to be significantly associated ($P < 0.05$) with birth weight and weaning weight on genes TG, ADPOD, CAST, and IGF-1. Interpretation of SNP associated with carcass traits revealed 13 SNP within TG, ADPOQ, IGF-1, and CAST genes significantly associated ($P < 0.05$) with hot carcass weight, back fat thickness, marbling score, and yield grade. Multiple markers were found to be significantly associated with multiple traits on TG, ADPOQ, IGF-1, and CAST genes, It is important to note that several markers were observed trending ($P < 0.10$) and should be considered in future research. Furthermore, a larger population size should be utilized to validate the significant association reported in the study herein.

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APPENDIX A: DNA EXTRACTION – SATURATED SALT PROCEDURE

Based on extraction procedures described in Miller et al., 1998. Nucl. Acids Res. 16: 1215.

Day 1: in 15 ml centrifuge tube

Add: 10-12 ml Lysis buffer (Appendix B) to 250 L white blood cell buffy coat; invert to mix

Spin: 7000 rpm for 10 minutes at 4 °C; aspirate supernatant from pellet

Add: 3 mls Digestion Buffer (Appendix B); shake vigorously to resuspend pellet

Add: 200 µl 10% SDS and 60 µL RNase A (10mg/ml); invert to mix; incubate for 1 hr at 37 °C with gentle shaking

Add: 25 µl Proteinase K (20mg/ml); invert to mix; incubate overnight at 37 °C with gentle shaking

Day 2:

Add: 1 ml Saturated NaCl; shake vigorously by hand for 15 seconds

Spin: 2800 rpm for 30 mins at 4 °C; transfer supernatant to new 15 ml tube

Add: 2 volumes of 100% Ethanol (stored in freezer); invert gently to mix

Remove: DNA with soft pipette; transfer DNA into 1.5 ml snap-cap tube

Spin: at 10 setting for 10 min. in refrigerated bench-top centrifuge; aspirate off most of ethanol

Add: 1 ml of 80% ethanol (keep on ice); vortex for 20 seconds; spin 5 minutes in refrigerated bench-top centrifuge; aspirate off most of ethanol

Leave tubes uncovered to allow pellet to dry overnight

Add: 350 µl Rehydration Buffer (Appendix B) to resuspend DNA

Read: on spectrophotometer

APPENDIX B: BUFFER SOLUTION LABORATORY PROTOCOL

LYSIS BUFFER (1L):

7.49g NH_4Cl

2.059g Tris-HCl

pH to 7.4

DIGESTION BUFFER (1L):

1.211g Tris-HCl

23.376g NaCl

0.744g EDTA

pH to 8.0

REHYDRATION BUFFER (1L):

1.21g Tris-HCl

0.37g EDTA

VITA

Malcolm Seth Mizell was born June of 1989 to parents Mickey and Karen in Baton Rouge, Louisiana. Seth graduated from Independence High School in 2007 and started his undergraduate studies at Louisiana State University in the fall of 2007. Seth graduated with a bachelor's degree in Animal, Dairy, and Poultry Sciences with a concentration in Dairy Production in May of 2011.

Seth began his Master of Science degree at Louisiana State University with major professor, Dr. Matthew Garcia. After Seth completes his Master of Science degree he hopes to pursue a career in the livestock pharmaceutical sales industry.