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A GENOMIC AND QUANTITATIVE EVALUATION OF MODERN CHAROLAIS SIRE
CALVES VERSUS MULTIGENERATIONAL ANGUS SIRE CALVES
FOR GROWTH AND CARCASS QUALITY AND COMPOSITION 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 Interdepartmental Program of
Animal and Dairy Sciences

by
Jennifer Lynn Bailey
B.S., Louisiana State University, 2010
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TABLE OF CONTENTS

ACKNOWLEDGEMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
ABSTRACT	vii
CHAPTER	
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	3
Hybrid Vigor	3
Selection Methods	4
Cattle Trends	5
Trends in the Angus and Charolais Breeds	5
Carcass Characteristics	7
Mixed Model Designs	10
Genetic Markers	11
Quantitative Trait Loci	12
SNP Chip Technology (Iplex)	13
Selective Genotyping	16
Marker Assisted Selection	16
Whole Genome Selection	17
Calpain Gene	19
Calpastatin Gene	20
III. EVALUATION OF SIRE BREED TYPE ON GROWTH AND CARCASS CHARACTERISTICS UTILIZING MULTIGENERATION ANGUS SIRE CALVES VERSUS CHAROLAIS SIRE CALVES	22
Introduction	22
Experimental Animals	23
Growth and Carcass Characteristics	24
Statistical Analysis	24
Results	25
Discussion	26
IV. AN SNP ASSOCIATION STUDY EVALUATING MODERN CHAROLAIS SIRE CALVES VERSUS MULTIGENERATIONAL ANGUS SIRE CALVES FOR GROWTH AND CARCASS TRAITS	36
Introduction	36
Experimental Animals	37
Growth and Carcass Characteristics	37
Candidate Gene Selection	38

SNP Selection	39
DNA Extraction	39
Statistical Analysis.....	39
Results	40
Discussion.....	52
V. SUMMARY	54
LITERATURE CITED	55
APPENDIX A: DNA EXTRACTION – SATURATED SALT PROCEDURE.....	62
APPENDIX B: BUFFER SOLUTIONS.....	63
VITA	64

LIST OF TABLES

4.1	SNP ID, forward and reverse primer sequences and allele substitution at SNP location utilized to obtain genotypes for 20 SNP located on the CAPN3 gene ...	41
4.2	SNP ID, forward and reverse primer sequences and allele substitution at SNP location utilized to obtain genotypes for 20 SNP located on the CAST gene	42
4.3	Level of significance, numbers of animals from each genotype, and S. E. for markers significantly associated with weaning weight and hip height	43
4.4	Single nucleotide polymorphisms significantly associated with weaning weight and hip height and least square means comparisons between reported genotypes.....	44
4.5	Level of significance, numbers of animals from each genotype, and S. E. for markers significantly associated with average daily gain	45
4.6	Single nucleotide polymorphisms significantly associated with average daily gain and least square means comparisons between reported genotypes.....	45
4.7	Level of significance, numbers of animals from each genotype, and S. E. for markers significantly associated with hot carcass weight.....	46
4.8	Single nucleotide polymorphisms significantly associated with hot carcass weight and least square means comparisons between reported genotypes.....	47
4.9	Level of significance, numbers of animals from each genotype, and S. E. for markers significantly associated with marbling score	48
4.10	Single nucleotide polymorphisms significantly associated with marbling score and least square means comparisons between reported genotypes.....	48
4.11	Level of significance, numbers of animals from each genotype, and S. E. for markers significantly associated with rib eye area.....	49
4.12	Single nucleotide polymorphisms significantly associated with rib eye area and least square means comparisons between reported genotypes.....	50
4.13	Level of significance, numbers of animals from each genotype, and S. E. for markers significantly associated with back fat.....	51
4.14	Single nucleotide polymorphisms significantly associated with back fat and least square means comparisons between reported genotypes	51

LIST OF FIGURES

3.1	Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of birth weight.....	27
3.2	Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of weaning weight.....	28
3.3	Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of hip height.....	29
3.4	Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of average daily gain in the feedlot.....	30
3.5	Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of hot carcass weight.....	31
3.6	Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of rib eye area	32
3.7	Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of back fat.....	33
3.8	Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of marbling score.....	34

ABSTRACT

The objective of this study is to evaluate the association of single nucleotide polymorphisms with growth, performance, and carcass quality and composition characteristics in a population of cattle consisting of multigenerational Angus sired calves and modern characteristic Charolais sired calves. A total of 132 calves were evaluated. Due to a limited sample population statistical difference was set at $p < 0.05$ and statistical trends were set at $p < 0.1$.

Mean birth weights were significantly higher for Charolais sired calves as compared to Angus sired calves. Significant differences were observed between the two breed types for mean weaning weights with Angus sired calves having heavier weaning weights as compared to Charolais sired calves. Charolais sired animals had significantly larger rib eye areas as compared to the Angus sired animals. A significant difference was observed between the two groups for back fat thickness with the Angus sired animals having a larger amount than the Charolais sired animals. Angus sired animals had significantly higher marbling scores as compared to the Charolais sired animals.

Eleven SNP on CAPN3 and two SNP on CAST were found to be associated with at least one of the traits observed. Two of these SNP were significantly associated with weaning weight. Three of these SNP were significantly associated with hip height. One SNP was significantly associated with average daily gain. Three of these SNP were significantly associated with hot carcass weight. One of the SNP was significantly associated with marbling score. One was significantly associated with rib eye area and two of these SNP were significantly associated with back fat thickness.

CHAPTER I INTRODUCTION

Identification and evaluation of molecular markers associated with economically important traits may prove to be a valuable tool that increases accuracy of selection in livestock species. Evaluation of single nucleotide polymorphisms located on candidate genes of known physiological function allows for the potential identification of SNP that are accounting for variability observed in the trait of interest. Several candidate genes have been associated with carcass quality and carcass composition traits, however the calpastatin gene (CAST) and the calpain III gene (CAPN3) have been the most frequently evaluated (Huang and Forsberg, 1998; Patel and Lane, 1999; Schnekkel et al., 2006).

The populations evaluated in this study consist of calves sired by Angus sires born between 1960 and 2006 and calves sired by Charolais sires with EPD's consistent with current breed trends. These two populations were selected due to the phenotypic differences expected between the offspring of the two sets of sires. A study by Williams and associates (2010) observed that the Charolais breed was larger in both total size and amount of muscle on the carcass and had less fat and marbling than the Angus breed (Williams et al., 2010). Traits evaluated in the current study include birth weight, weaning weight, hip height, hot carcass weight, average daily gain, rib eye area, back fat thickness, and marbling score.

The calpastatin gene (CAST) has been reported to be associated with meat tenderness due to its role as a regulatory inhibitor of calpains (Killefer and Koohmarie, 1994). An increase in calpastatin activity may reduce the muscle protein degradation

rate, thus promoting increased growth and enhancing the efficiency of growth (Huang and Forsberg, 1998). A previous study reported associations between the CAST gene and fat yield, rib eye area, and lean yield (Schenkel et al., 2006).

The calpain gene (or CAPN3) has been shown to be associated with protein degradation (Huang and Forsberg, 1998) and the differentiation of preadipocytes to adipocytes (Patel and Lane, 1999). This is important due to previous reports that the skeletal muscle specific (Spencer et al., 2002) CAPN3 is involved in the breakdown of myofibrillar proteins and is closely related to meat tenderness (Schenkel et al., 2006).

The objective of the current study was two-fold. The first objective was to evaluate the growth differences between the two sire breeds on crossbred calves, secondly, the evaluation of single nucleotide polymorphisms in the CAST and CAPN3 genes in a population comprised of multigenerational Angus sired animals and Charolais sired animals with growth and efficiency traits as well as carcass quality and composition traits.

CHAPTER II REVIEW OF LITERATURE

Hybrid Vigor

The term hybrid vigor is used to describe the increased vigor and performance of the crossbred offspring for a variety of traits and is used interchangeably with the term heterosis to describe the performance observed in the crossbred, or hybrid, progeny (Livesay, 1930; Cundiff, 1970). Beef cattle producers have utilized crossbreeding systems to take advantage of hybrid vigor observed due to crossing different breeds of cattle. Crossbreeding animals of different breed and biological type produces F₁ progeny. It has been documented that F₁ progeny express the highest level of hybrid vigor (Cundiff, 1970) due to an increase in the heterozygosity of the allelomorphic pairs of genes (Livesay, 1930).

Utilization of purebred sires on crossbred cows allows for favorable traits of the parent breeds to be more prevalent in the progeny. Thus, an efficient method of maintaining heterosis through generations is the use of multi-breed rotational crossbreeding systems (Koch et al., 1985). Multi-breed rotational crossbreeding systems utilize purebred sires and crossbred dams. This system introduces sires of different breeds in sequence. Purebred sires are bred to crossbred cows whose breed composition consists of the lowest proportion to the current sire breed type. Any benefit of parent breeds due to interaction between loci, or epistasis is retained at a higher level than if both parents were crossbred (Koch et al., 1985).

A study conducted by Gregory and associates (1994) observed that retained hybrid vigor in future generations of crossbred cattle is proportional to retention of

heterozygosity. This finding supports the hypothesis that dominance effects of genes account for a large amount of heterosis. Koch and associates (1985) reported that individual heterosis is significant for increased birth weight, weaning weight, post weaning weight, carcass weight, fat cover, and rib eye area. Sacco and associates (1991) reported that the average heterosis retained in later generations of crossbred calves is significant for increased weaning weight and increased weaning height.

Selection Methods

Methods of selection for economically important traits in livestock have improved dramatically over the past five decades. The ability to select animals using performance test data, EPDs, and molecular information has been incorporated into many beef production schemes as a method to increase the accuracy of selection. Prior to these technologies selection was primarily based on visual characteristics and individual performance (Garrick and Golden, 2009).

Expected progeny differences (EPDs) have been utilized to evaluate the potential breeding value of purebred animal. EPDs are calculated by analyzing the phenotypic performance of an individual's offspring and comparing those offspring to the base herd average for the breed. EPDs for specific traits are often evaluated when determining a breeding objective for a population (Harris and Newman, 1994). EPDs for each trait can be weighted on its economic value and added together to better its usefulness in index selection (Garrick and Golden, 2009). The development of best linear unbiased prediction (BLUP) and improvements in computing capabilities improved the ability to collect, combine, and analyze information on relatives to generate EPD's (Harris, 1998).

Cattle Trends

Changes in the average growth rate, size and composition have occurred in the beef cattle industry over the past 50 years. Previous research by Berg and Walters (1983) reported that from 1958 to 1983 fat yield at slaughter decreased and growth rate and mature size increased. It was also reported that breeds that tended to fatten early (British breeds) typically showed higher subcutaneous fat to intramuscular fat ratios than continental breeds (Berg and Walters, 1983).

In 1966, a study evaluating three British breeds reported an average carcass weight of 252 kg, an average rib eye area of 69 cm², an average back fat thickness of 1.24 cm, an average marbling score of 6.5 (between high small and low modest), and an average 252-day average daily gain of 0.820 kg (Swiger et al., 1966). However, Wheeler and associates (1996; 2005) reported significant improvements from the previously reported studies. From 1996-2005, average live weight increased by 78 kg, average hot carcass weight increased by 42 kg, average dressing percent decreased 1%, average adjusted fat thickness decreased by 0.08 cm, average rib eye area increased by 10.7 cm², and average marbling score increased by 20.4. Information reported by the Focus on Feedlots program (1990-2011) states that average daily gain has increased by ~0.42 lb/head/day and market weight has increased by ~160 pounds (www.asi.ksu.edu).

Trends in the Angus and Charolais Breeds

Dramatic improvements in the beef cattle industry have been made over the last five decades. Specifically, the Angus breed has made major increases in traits such as birth weight, weaning weight, yearling weight, average daily gain, mature weight,

carcass weight, marbling score, rib eye area, and back fat thickness

(<http://www.angus.org/Nce/GeneticTrends.aspx>). The Angus breed has evolved from a small framed maternal breed into a breed with more paternal breed characteristics.

Wilson and associates (1993) reported genetic trends from 1969-1989 noting age at slaughter decreased ~20 days and carcass weight at slaughter has increased ~100 pounds. It was further reported that as carcass weight has increased so has the size of the longissimus area. Carcass weight in the Angus breed has increased by ~13 kg and longissimus area has increased by ~3.2 cm². Marbling score has decreased but back fat thickness has had a greater decrease over the years. Marbling score has decreased by ~0.1 while back fat thickness has decreased by ~1.5 cm (Wilson et al., 1993).

When evaluating paternal breeds such as the Charolais, dramatic improvements have been made as well. The Charolais breed has reported a five pound decrease in birth weight over the last 21 years. Furthermore, an increase in production, efficiency and carcass traits including a 32 pound increase in weaning weight and a 78 pound increase in yearling weight. Increases in carcass quality and composition traits included an increase in carcass weight (3.7-15.0), rib eye area (0.02-0.20), and slight increases in back fat thickness (-.003-0.000) and marbling score (0.01-0.02) are also reported (<http://www.charolaisusa.com/epdstats.html>).

A study conducted by Williams and associates (2010) utilized production and carcass trait data reported in studies from 1976 to 1996 to calculate breed and heterosis effects on the traits of interest. It was observed that the Charolais breed consistently had higher birth weights, weaning weights and post-weaning gains when compared to maternal breeds of cattle. The Charolais breed also displayed higher carcass weights

and larger longissimus muscle area when compared to maternal breeds of cattle. Due to the paternal nature of this breed, a lower back fat thickness and lesser amount of marbling is also shown when compared to the Angus breed (Williams et al, 2010).

Cundiff and associates (1998) reported on data from the Cycle IV of the Germplasm Evaluation program at MARC and observed that Charolais sired animals had heavier birth weights and weaning weights than Angus sired animals. A study conducted by Arango and associates (2002) utilized growth trait data collected on animals in Cycle I of the Germplasm Evaluation program at MARC. It was observed that Charolais sired cows consistently had taller hip heights and heavier weights than Angus sired cows. Thallman and associates (1999) utilized growth trait data from Cycle IV of the Germplasm Evaluation program. It was observed that Charolais sired heifers had heavier weaning weights, heavier yearling weights, and had significantly taller hip heights than Angus sired heifers.

A study conducted by Gregory and associates (1994) reported that Charolais sired animals had higher average daily gains, heavier carcass weights, and lower marbling scores than Angus sired animals. DeRouen and associates (1992) performed a study evaluating four generations of crossbreeding effects on carcass traits. In this study, it is reported that Charolais animals had heavier hot carcass weights and larger rib eye areas. It was also observed that Angus animals had greater back fat thickness and marbling scores.

Carcass Characteristics

Carcass quality and composition traits are evaluated in meat animals post-harvest to determine the quality and yield of the final carcass product. Carcass

composition traits that are evaluated include hot carcass weight (HCW), adjusted fat thickness, longissimus muscle (LM) area, yield grade (YG), dressing percentage, kidney, pelvic, heart (KPH) fat, meat tenderness, and retail product yield (RPY), fat, and bone yield. Carcass quality traits that are evaluated include marbling score, quality grade (QG), meat color, and meat tenderness.

Carcass yield traits give information about how much of the carcass can potentially be made into retail product. Hot carcass weight is measured by immediately weighing the carcass after the internal organs are removed. HCW gives information about how much of the carcass was internal organs that are unable to be made into retail product as well as how much of the carcass can now be made into retail product (Koch et al., 1976). Dressing percentage is calculated as the ratio between live weight and hot carcass weight. A higher dressing percentage is preferred because the internal organs are unable to be made into lean retail product (Wheeler et al., 2005). Cold carcass weight is measured after a 24 hour chill period. Cooler shrinkage is the difference between cold and hot carcass weight and gives estimation on the loss of moisture during the cooling process (Koch et al., 1976).

Carcass traits measuring fat yield information about how much excess fat is on a carcass as well as the potential meat quality. Adjusted fat thickness is the distance measured at the 12th rib between the longissimus muscle and the edge of the fat layer. Adjusted fat thickness is useful in interpreting how much excess fat is on the carcass that must be removed for the production of acceptable retail product. Larger amounts of external fat are associated with more marbling, which is desirable and has been documented to contribute to tenderness (Wheeler et al., 2005). Kidney, pelvic, heart

(KPH) fat is the amount of fat in the visceral area measured as a percentage of carcass weight. KPH is a factor used to determine yield grade (Koch et al., 1979) and a higher percentage of KPH is associated with healthier cattle in the feedlot (Gardner et al., 1999).

Longissimus muscle area is also described as rib eye area (REA) and is measured by cutting between the 12th and 13th rib. Longissimus muscle area measures the area of the longissimus muscle in cm². A larger longissimus muscle is desirable as it contributes to the more valuable retail product and indicates a more heavily muscled carcass (Wheeler et al., 2005).

Many of the previously described traits also contribute to the calculation of yield grade. Yield grade is an estimate of the amount of final retail product available on the carcass. A USDA yield grade (1-5) is assigned based on the evaluation of HCW, adjusted fat thickness, REA, and KPH percent. A USDA yield grade of 1, the most desirable grade, describes a carcass with a very thin layer of fat over the carcass with the lean easily visible through the fat on many areas of the carcass. A USDA yield grade of 3 describes a carcass completely covered in fat with lean only visible through the fat on the neck and the lower extremities. A USDA yield grade of 5 describes a carcass completely covered in fat with no lean visible (USDA, 1997).

Quality grade is determined by evaluating the following carcass traits. Marbling score is a carcass quality trait determined by comparing the amount of intramuscular fat in the longissimus muscle to a USDA reference chart with ten degrees of marbling ranging from very abundant to practically devoid (USDA, 1997). A USDA quality grade (prime, choice, select, etc.) is assigned based on the evaluation of marbling score and

maturity of the animal (USDA, 1997). Quality grade is a combined evaluation of factors that influence the palatability of meat including marbling, skeletal maturity, lean maturity, color, texture, and firmness of lean (Koch et al., 1976).

Another vital component to overall meat palatability is meat tenderness. Meat tenderness is measured using Warner-Bratzler shear force which is a process that utilizes small samples of cooked steak and shears the sample perpendicular to the muscle fiber. Warner-Bratzler shear force is measured in kilograms and lower values indicate more tender meat (Wheeler et al., 1998).

Mixed Model Designs

Mixed model designs are useful statistical approaches capable of analyzing complex data and data with correlated effects (Xu and Yi, 2000). Mixed model designs contain both fixed and random effects and are capable of analyzing data existing in a grouping structure. Fitting a mixed model involves using restricted maximum likelihood (REML) to estimate fixed effects and covariance matrixes and using BLUP to predict random effects (Wand, 2003).

Manipulation of variables in mixed models can be utilized to analyze different types of data (Wand, 2003). Mixed models are advantageous for finding differences when executing studies searching for associations between genetic markers and quantitative traits (Sun et al., 2010). The mixed model has been utilized in numerous studies to evaluate the associated between SNP markers and carcass quality and composition traits (Casas et al., 2005; Casas et al., 2007) such as meat tenderness (White et al., 2005). Mixed models have also been used in studies evaluating

associations between SNP markers and the disease keratoconjunctivitis (Garcia et al., 2010) as well as fat deposition and meat tenderness (Stone et al., 2005).

Genetic Markers

Genetic markers are variants in DNA sequences at the molecular level. The main types of molecular markers utilized in genomics research are restriction fragment length polymorphisms (RFLPs), single nucleotide polymorphisms (SNPs), insertions, deletions (INDELs), and microsatellite markers (Vignal et al., 2002).

RFLPs are segments of DNA that have restriction/cleavage sites that are recognized by bacterial restriction enzymes. The restriction sites in DNA are specific nucleotide sequences and differ for different restriction enzymes. The restriction enzyme recognizes a specific sequence of DNA and cuts any strand of DNA with the recognized sequence. Restriction fragment length polymorphisms can differ in size and are separated using agarose gel electrophoresis. This allows for the identification of different alleles at a specific locus in a population due to the size differences among alleles (Montaldo and Meza-Herrera, 1998).

Microsatellites are tandem repeats within DNA sequence at a specific locus. These repeats are composed of one to six base pairs the most common being dinucleotides. Microsatellites are found throughout the genome although they are less frequently found in coding regions (Li et al., 2002). The distribution of microsatellites throughout the genome and the high amount of variation that can occur among microsatellites makes them excellent genetic markers. PCR and electrophoresis techniques are used to determine the components and length of microsatellite markers (Ihara et al., 2004).

The construction of genetic maps in human and animal species was executed using mainly RFLPs until microsatellite markers replaced them as the main tool for genetic mapping. This change occurred largely due to the high number of alleles present at a microsatellite locus. The large number of alleles at a single microsatellite locus increases heterozygosity values and reduces the required number of reference families required to build a genetic map (Vignal et al., 2002).

Single nucleotide polymorphisms (SNP) are defined as a single nucleotide base change at a specific locus of the DNA sequence. Single nucleotide polymorphisms are described as bi-allelic markers because only two nucleotides are typically possible alternatives. For genomic nucleotide sequences to be designated as an SNP, the least recurrent nucleotide should have a frequency of at least one percent deeming it significantly present. Using SNPs as a genetic marker gives a high density of markers permitting more loci to be evaluated in studies. The benefits of SNPs as genetic markers have led to the development of SNP chips which are capable of evaluating thousands of loci at a time (Vignal et al., 2002; <http://www.illumina.com>).

Quantitative Trait Loci

Quantitative trait loci (QTL) are defined as a specific region on a chromosome that potentially harbors genes that have a significant effect on quantitative traits (Paterson et al., 1990). The phenotypic differences observed can be attributed to allelic differences of genes as well as interactions among alleles/genes in differing regions of chromosomes and on different chromosomes (Lander and Botstein, 1989).

The ability to map QTLs with relatively large phenotypic effects aids in the understanding in the variability of performance for specific quantitative traits (Paterson

et al., 1990). The traditional approach of QTL mapping utilizes extreme phenotypes of different groups of progeny. Two assumptions were utilized in the traditional approach. The first assumption was that individuals with similar phenotypes share the same alleles in specific regions of the chromosome. The second assumption was that the QTLs between individuals with dissimilar phenotypes have differing alleles in the specific region. Lander and Botstein (1989) suggested combining interval mapping using complete RFLP linkage maps and selective genotyping. Interval mapping utilizes the method of maximum likelihood to determine the most likely position of the QTL affecting the phenotype. This method has been shown to increase the power and efficiency of QTL mapping (Lander and Botstein, 1989).

Many factors can affect the accuracy of QTL mapping procedures such as heritability of the trait, the number of genes influencing the trait, the number of marker loci employed and the marker distribution over the genome. Gene interactions and distribution over the genome can influence QTL mapping accuracy so larger numbers of markers per chromosome are required to increase the efficiency of QTL mapping. Furthermore, the type and size of the population studied as well as linkage information may result in accuracy issues (van Ooijen, 1992).

SNP Chip Technology (Iplex)

An SNP bead chip can be defined as a silicon wafer with many small wells (indentations) on its surface in which beads with attached primers can be placed (Fan et al., 2010). Illumina has constructed substrates with tens of thousands of these microwells across their surfaces. Each microwell contains a different probe sequence

that is covalently attached to the well and through a hybridization procedure the probe ID can be determined (Shen et al., 2005).

SNP chip technologies take advantage of the bi-allelic nature of SNP markers as well as the high density of these markers in the genome. A method of utilizing SNP chips includes DNA fragmentation and amplification of fragmented DNA using PCR. The amplified sample is then hybridized to a SNP chip containing probes for each SNP marker. The probes reveal which SNP marker polymorphism is present in the sample (Carvalho et al., 2007).

SNP chips have the potential to increase the accuracy of whole genome selection and may be of use in both purebred and crossbred populations. Due to artificial selection some markers that are physically far apart may be in high linkage disequilibrium. Linkage disequilibrium is the association of the same multiple alleles/markers with a specific trait. In order to increase genotyping accuracy, the extent of linkage disequilibrium must be determined in a population to determine the appropriate SNP density for a particular study. For example, a study consisting of a population with a smaller amount (increased physical distance) of linkage disequilibrium between SNP and the loci being analyzed would benefit by using a SNP chip with a higher density of SNPs (Fan et al., 2010).

SNP chips have a variety of potential applications in genomic selection including whole genome association studies and marker assisted selection. SNP chips have been utilized in constructing high-resolution linkage maps and linkage disequilibrium mapping, or locating the markers in association with a trait. SNP chips increase the efficiency of mapping studies due to the ability they have to analyze thousands of SNP

in a specific locus or across the entire genome. The major advantage of SNP chips is their utilization in whole genome association and marker assisted selection studies. These studies have the capability to identify regions of genomes significantly influencing quantitative traits or specific SNP associated with a trait of interest in a more efficient method than has traditionally been utilized (Fan et al., 2010).

A tool that has been and can be used in these types of studies is Illumina's BeadArray technology. This BeadArray technology (illumina.com) utilizes a high number of samples to perform high-throughput genotyping (Fan et al., 2010). A bead array has many unique bead types which each have different probe sequences (Shen et al., 2005). It is designed to use either single-base extension or allele-specific primer extension. The SNPs used in genomic analysis studies consist of SNP which have been discovered through whole genome sequencing studies or haplotype mapping studies and can be found through the dbSNP database on NCIB or private collections (Fan et al., 2010).

Illumina's Bovine SNP 50 Beadchip utilizes 54,609 SNP probes. This particular array is capable of being utilized in genome-wide selection, identification of QTL, evaluation of genetic merit, and comparative genetic studies due to the uniform distribution of the SNP probes throughout the bovine genome (VanRaden et al, 2009; Wiggans et al., 2009; Wiggans et al., 2010). The average space between probes is 49.4 kilobases which is within the suggested linkage disequilibrium in cattle providing high quality data (<http://www.illumina.com>).

Selective Genotyping

Selective genotyping is a genotyping strategy described by Lander and Botstein (1989) in which individuals that are phenotypic outliers are selected for genetic evaluation of a trait of interest. Selective genotyping reduces the number of individuals necessary to be genotyped when detecting quantitative trait loci (Lander and Botstein, 1989). When selective genotyping is utilized to detect QTLs, the power to locate a rare allele of a large effect is increased because there is a higher likeliness of it being present in the population utilized for selective genotyping (Slatkin, 1999). This method can also be utilized to improve the precision of linkage disequilibrium mapping (Stella and Boettcher, 2004).

Marker Assisted Selection

Marker assisted selection (MAS) is a tool that utilizes genetic markers associated with economically important traits to identify individuals predisposed for certain phenotypic characteristics. Marker assisted selection is most advantageous when the marker accounts for a large amount of the variation observed in the trait. Markers linked to QTL are used because many economically important traits are quantitative in nature. Utilizing markers linked to QTL enables the detection of individuals with favorable combinations of markers or gene variants affecting quantitative traits (Davis and DeNise, 1998).

Utilizing marker assisted selection may be capable of doubling the response to selection in the first generation for a particular trait as compared to the response when using only phenotypic selection. The increased level of performance is also dependent the number of markers, the heritability of the trait and the interactions of markers

influencing quantitative traits (Davis and DeNise, 1998). The implementation of new technologies in combination with previous methods can assist producers in making economically sound judgments and increase accuracy of selection (Harris, 1998).

The benefits of MAS have previously been described. Rothschild and associates (1996) reported that the ESR, estrogen receptor, locus has been shown to have a significant influence on litter size in pigs. This information was validated by Short and associates (1997) in a study evaluating the application of marker assisted selection. Short and associates (1997) reported that the application of MAS lead to an increase of the favorable ESR allele causing a significant increase in litter size.

The development of markers to be utilized in marker assisted selection is most valuable when used in large populations. Once markers accounting for the greatest amount of variability in a quantitative trait are identified, the effectiveness of MAS programs can be increased by selecting animals with favorable combinations of specific markers which influence the trait (Davis and DeNise, 1998).

Whole Genome Selection

Whole genome selection (WGS) is a selection approach using all markers significantly associated with a trait. Unlike marker assisted selection which utilizes a low number of associated markers, whole genome selection will utilize all markers in the genome significantly associated with a trait increasing the accuracy of animal selection for breeding and production purposes. Analysis of these markers yields information pertaining to the phenotypic effects of markers for a wide range of traits throughout the genome (Goddard and Hayes, 2007). While whole genome selection uses all markers throughout the genome associated with a trait, it puts greater emphasis on regions with

the largest effects. It utilizes genotypes of thousands of SNP some of which have smaller effects allowing WGS to account for more genetic variation (Thallman, 2009). Utilizing the information observed in the variation of DNA sequence should allow more accurate prediction of breeding values for numerous traits (Goddard and Hayes, 2007).

Benefits of whole genome selection include the ability of the marker panel to locate all the genetic variance for a trait and to estimate the marker effect on a population basis rather than within a family. The accuracy of whole genome selection increases with increased sample size. The large reference population required for accuracy as well as the involvement of multiple cattle breeds does present a challenge to whole genome selection. The development of dense SNP arrays allows for the improvement of accuracy in whole genome selection. Other benefits of whole genome selection include the ability to select animals at a very young age based on genetic markers instead of through the longer time period required for progeny testing. This could allow for faster genetic improvement towards performance goals in each generation interval (Goddard and Hayes 2007).

Three population types are utilized in WGS. Information from training populations is used to estimate the effects of markers on a trait and provides a panel of markers of interest for a trait. The validation population consists of animals not included in the training population. Individuals in this population are genotyped and selected based on the marker effects estimated in the training population (Garrick, 2011). The data from the validation population is analyzed to determine how much the markers in the training population predict the phenotypic data. The third is the application population in which

individuals will be assigned molecular breeding values based on results from the validation population (Thallman, 2009).

Barendse and associates (2007) performed a whole genome association study in multiple breeds of cattle and reported finding SNPs with significant associations with feed conversion efficiency. Another whole genome association study conducted by Bolormaa and associates (2011) reported SNPs significantly associated with residual feed intake, average daily gain, daily feed intake, birth weight, and hip height. SNP significantly associated with birth weight, weaning weight, and yearling weight have also been reported by Snelling and associates (2010) and many of the SNP significantly associated with growth overlap QTL linked to growth traits.

Calpain Gene

The calpain III (CAPN3, p94) gene is located on *Bos taurus* autosome 10. The genomic region of the CAPN3 gene spans 57,439 base pairs from 37,829K to 37,885K base pairs and the gene contains 24 exons and 23 introns (<http://www.ncbi.nlm.nih.gov>). The CAPN3 gene is associated with calpains which are calcium-activated cytoplasmic cysteine proteases and are involved in a variety of calcium regulated cellular processes. Calpains are involved in the early steps of the process of differentiation of preadipocytes to adipocytes (Patel and Lane, 1999) and mediate the process of muscle-cell proteolysis or protein degradation which initiates the breakdown of individual myofibrillar proteins (Huang and Forsberg, 1998). Myofibrillar protein breakdown (Schenkel et al., 2006) and increased fat laydown such as intramuscular fat have both been correlated to meat tenderness (Wheeler et al., 2010). The observation that calpain III is skeletal muscle specific and modulates signal

transduction around skeletal muscle infers that it has a role in muscle differentiation and development (Spencer et al., 2002). Recently, it has been suggested that calpain III may have an effect on meat tenderness due to the skeletal muscle specificity (Ilian et al., 2001). Café and associates (2010) reported that a SNP in the CAPN3 gene was associated with less shear force. A study conducted by Robinson and associates (2012) observed an association between SNP at the CAPN3 gene and meat tenderness. As such, the gene that codes for calpain III (CAPN3), located on bovine chromosome 10, is of great interest as a candidate gene for effects on growth and meat tenderness (Barendse et al., 2008).

Calpastatin Gene

The calpastatin (CAST) gene is located on *Bos taurus* autosome 7. This genomic region spans 171,144 base pairs from 98,444K to 98,581K base pairs and the gene contains 35 exons (<http://www.ncbi.nlm.nih.gov>) and 34 introns (Raynaud et al., 2005). The CAST gene is associated with calpastatin which is a specific inhibitor of calpains, and as such plays a regulatory role in muscle-cell proteolysis and meat tenderness. Previous studies have observed that as postmortem calpastatin activity is increased protein degradation is reduced leading to reduced meat tenderness (Killefer and Koohmaraie, 1994). A study by Schenkel and associates (2006) reported that an allele for a CAST SNP was associated with increased tenderness but tended to increase fat yield and decrease rib eye area and lean yield. Café and associates (2010) reported an association between an SNP in the CAST gene and greater rib fat. Café and associates (2010) also reported that favorable CAST alleles were associated with decreased shear

force. A study by Robinson and associates (2012) found significant associations between SNP on the CAST gene and meat tenderness.

Previous studies have described a decrease in myofibrillar protein breakdown is due to a reduction in calpain activity and an increase in calpastatin activity and vice versa (Ilian et al., 2001). The potential effect of these associations resulting in decreased myofibrillar protein breakdown would be decreased tenderness. Calpastatin may also have an effect on the promotion of animal growth and the enhanced efficiency of this growth (Huang and Forsberg, 1998). The CAST gene may prove to be a valuable candidate gene for the evaluation of growth and meat tenderness (Schenkel et al., 2006).

CHAPTER III

EVALUATION OF SIRE BREED TYPE ON GROWTH AND CARCASS CHARACTERISTICS UTILIZING MULTIGENERATIONAL ANGUS SIRE CALVES VERSUS CHAROLAIS SIRE CALVES

Introduction

Dramatic improvements in the beef cattle industry have been made over the last five decades. Specifically, the Angus breed has evolved from a small framed maternal breed to a breed with more paternal characteristics. Major increases have been observed in traits such as birth weight, weaning weight, yearling weight, and average daily gain. Genetic trends show a 3.8 pound increase in birth weight and a 54 pound increase in weaning weight (<http://www.angus.org/Nce/GeneticTrends.aspx>). Likewise, the Charolais breed, classified as a paternal breed, has also seen dramatic improvements in production traits. The Charolais breed has reported a five pound decrease in birth weight over the last 21 years and a 32 pound increase in weaning weight (<http://www.charolaisusa.com/pdf/2012/09.07/PhenotypicTrendTables.pdf>).

A study conducted by Williams and associates (2010) utilized production and carcass trait data reported in studies from 1976 to 1996. It was observed that the Charolais breed consistently had higher birth weights, weaning weights and post-weaning gains when compared to maternal breeds of cattle (Williams et al, 2010). More specifically, studies evaluating production traits have reported that Charolais sired animals have heavier birth weights (Cundiff et al., 1998), weaning weights (Cundiff et al., 1998; Thallman et al., 1999), hip heights (Arango et al., 2002; Thallman et al., 1999), and average daily gains (Gregory et al., 1994) than Angus sired animals. Previous studies evaluating carcass traits have reported that Charolais animals have higher average daily gain (Gregory et al., 1994), heavier hot carcass weights (Gregory

et al., 1994; DeRouen et al., 1992), larger rib eye areas, lower back fat thickness (DeRouen et al., 1992), and lower marbling scores (Gregory et al., 1994; DeRouen et al., 1992) than Angus animals.

The objective of this study was to test the effect of sire breed type on growth, performance, and carcass characteristics on a population consisting of Charolais sired calves and multigenerational Angus sired calves.

Experimental Animals

The population utilized in this study consisted of one hundred thirty-two animals sired by purebred Angus or Charolais sires mated to crossbred dams. The dam lines were comprised of one of four crossbred lines from the Germplasm Evaluation VIII population described in previous studies (Wheeler et al., 2005). The Angus sires utilized in the current study consisted of multigenerational sires born between 1960 and 2006. Dams had estrous synchronized via a previously described procedure (Wilson et al., 2010) and multigenerational Angus bulls were mated to the GPE VIII female population via artificial insemination. Upon completion of artificial insemination, Charolais sires were utilized as cleanup bulls for 67 days to increase pregnancy rates in the GPE VIII cows. The Charolais bulls were modern in their phenotype and contained modern EPD's typically utilized for a terminal herd sire.

All calves were born between January 15 and April 23, 2010 at the LSU AgCenter Central Research Station in Baton Rouge, LA. Calves were managed at this facility until weaning, or approximately six months of age. The population consists of 86 multigenerational Angus sired animals and 46 Charolais sired animals. Animals were nominated for the Louisiana calf to carcass program after a 45 day preconditioning

program and vaccination program that was established by the Louisiana calf to carcass program. Animals were then sent to either Hitch feedlot or Buffalo feedlot until a final harvest weight was obtained.

Growth and Carcass Characteristics

Growth and performance traits were collected at the Central Research Stations in Baton Rouge, LA and included birth weight (BW), weaning weight (WW), and hip height (HH). However, upon leaving the research station data was collected by the calf to carcass program included weight post shipping (shrinkage), days on feed, finishing weight and average daily gain (ADG). Animals were finished at either Hitch feedlot or Buffalo feedlot until a desired harvest weight of over 1200 lbs was reached. All animals were then transported to a commercial packing plant for harvest and subsequent carcass quality and composition trait measurement recording. Carcass quality and composition measurements that were collected included hot carcass weight (HCW), yield grade (YG), rib eye area (REA), back fat thickness (BF), marbling score (MARB) and quality grade (QG).

Statistical Analysis

The analyses conducted in this study were performed using the mixed procedure of SAS as previously described by White and associates (2005). Fixed effects include sire breed and sex. Random effects included the traits birth weight, weaning weight, hip height, average daily gain, hot carcass weight, back fat thickness, rib eye area, and marbling score. The LSMEANS function of SAS was utilized to conduct means separation analyses. Potential differences in performance for birth weight, weaning weight, hip height, hot carcass weight, average daily gain, back fat thickness, rib eye

area, and marbling score were evaluated between the multigenerational Angus sired calves and the Charolais sired calves.

Results

When analyzing the trait birth weight, Charolais sired calves had a mean of $43.0025 \text{ kg} \pm 0.9359 \text{ kg}$ which was significantly ($p < 0.05$) heavier than the Angus sired calves mean birth weight of $37.8011 \text{ kg} \pm 0.6845 \text{ kg}$ (Figure 3.1). A significant difference ($p = 0.05$) was detected for weaning weight in which Angus sired calves had a mean weaning weight of $260.90 \text{ kg} \pm 3.8752 \text{ kg}$ which was significantly heavier than the mean weaning weight of $247.97 \text{ kg} \pm 5.3573 \text{ kg}$ for the Charolais sired calves (Figure 3.2). Rib eye area measurements were significantly ($p = 0.05$) different between the two groups with Charolais sired animals having a mean rib eye area of $88.1353 \text{ cm}^2 \pm 1.3606 \text{ cm}^2$ which was significantly larger than the Angus sired animals mean rib eye area of $84.7934 \text{ cm}^2 \pm 1.0129 \text{ cm}^2$ (Figure 3.6). When analyzing the trait of back fat thickness, Angus sired animals had a mean back fat thickness of $0.5159 \text{ in} \pm 0.01985 \text{ in}$ in which was significantly ($p < 0.05$) more than the Charolais sired animals mean back fat thickness of $0.3757 \text{ in} \pm 0.02667 \text{ in}$ (Figure 3.7). A significant difference ($p < 0.05$) was detected for marbling scores in which Angus sired animals had a mean marbling score of 414.17 ± 7.9944 which was significantly higher than the Charolais sired animals mean marbling score of 380 ± 10.3208 (Figure 3.8). When evaluating hip height, no significant differences were observed between the Angus sired calves mean hip height of $17.5105 \text{ cm} \pm 0.08379 \text{ cm}$ and the Charolais sired calves mean hip height of $17.5678 \text{ cm} \pm 0.1158 \text{ cm}$ (Figure 3.3). No significant differences were observed between the Angus sired calves mean average daily gain of $1.5375 \text{ kg/day} \pm 0.02852 \text{ kg/day}$ and the mean

average daily gain of Charolais sired calves which was $1.5211 \text{ kg/day} \pm 0.03734 \text{ kg/day}$ (Figure 3.4). When evaluating the trait hot carcass weight, no significant differences were observed between the Angus sired calves mean hot carcass weight $352.38 \text{ kg} \pm 3.4481 \text{ kg}$ and the Charolais sired calves mean hot carcass weight $353.68 \text{ kg} \pm 4.7146 \text{ kg}$ (Figure 3.5).

Discussion

The objective of this study was to test the effect of sire breed type on growth, performance, and carcass characteristics on a population consisting of modern Charolais sired calves and multigenerational Angus sired calves. Results indicated that Charolais sired animals had significantly greater birth weights than multigenerational Angus sired animals and were validated by both Cundiff and associates (1998) and Williams and associates (2010). The significantly heavier weaning weights observed in the multigenerational Angus sired calves contradict the results reported by Cundiff and associates (1998) and Williams and associates (2010). These studies reported that Charolais sired animals displayed higher weaning weights than maternal breeds of cattle indicating the multigenerational Angus sires utilized in this study were more paternal than the base average of the breed.

When evaluating carcass quality and composition traits, the current study reported that the Charolais sired calves had a larger rib eye area than their multigenerational Angus sired counterparts. An association between Charolais animals and larger rib eye areas was also observed by Williams and associates (2010) and DeRouen and associates (1992). Results also indicate multigenerational Angus sired animals had significantly higher amounts of marbling in the longissimus muscle than

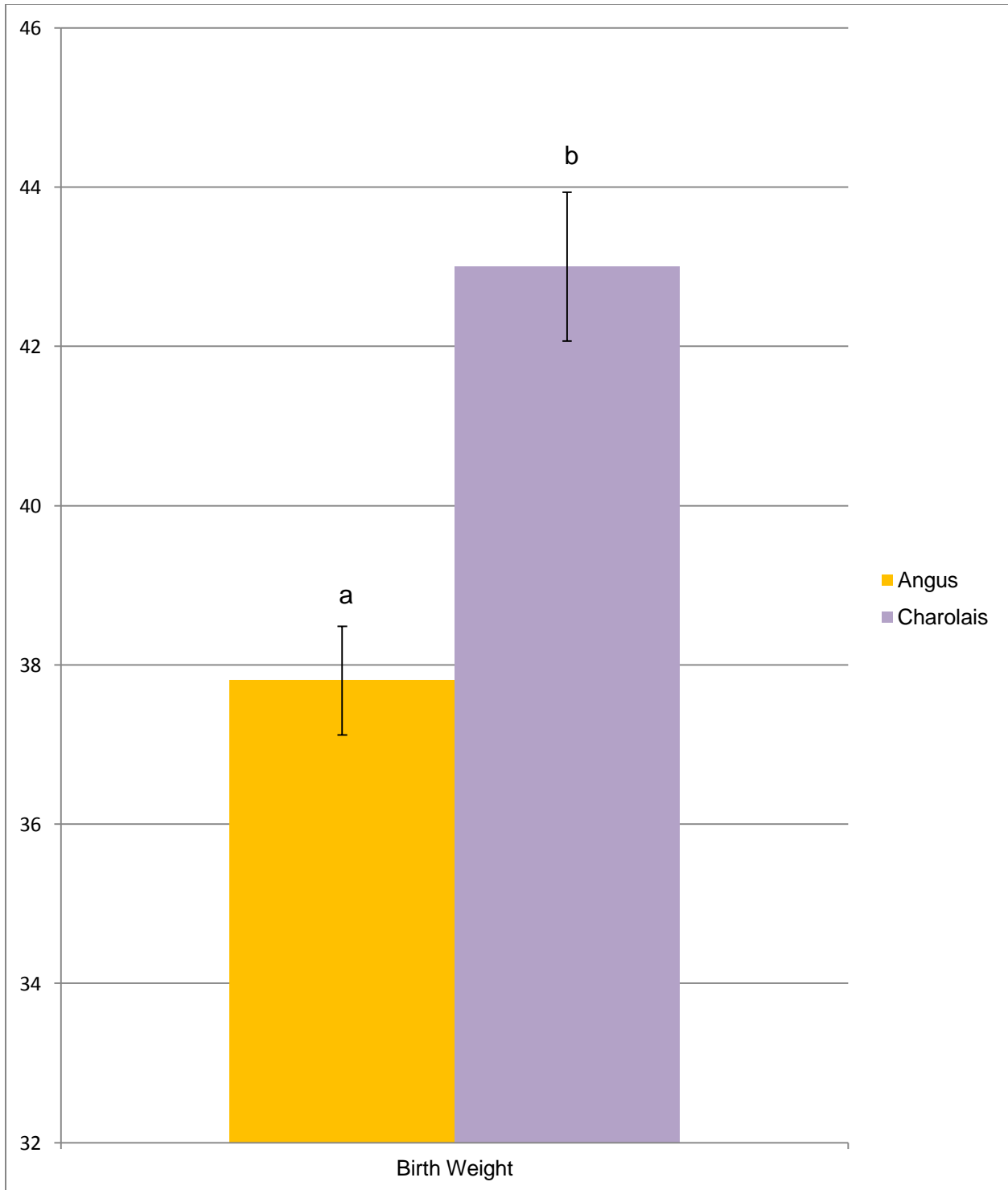


Figure 3.1: Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of birth weight.

^{a, b} Superscript indicates significance at $p < 0.05$

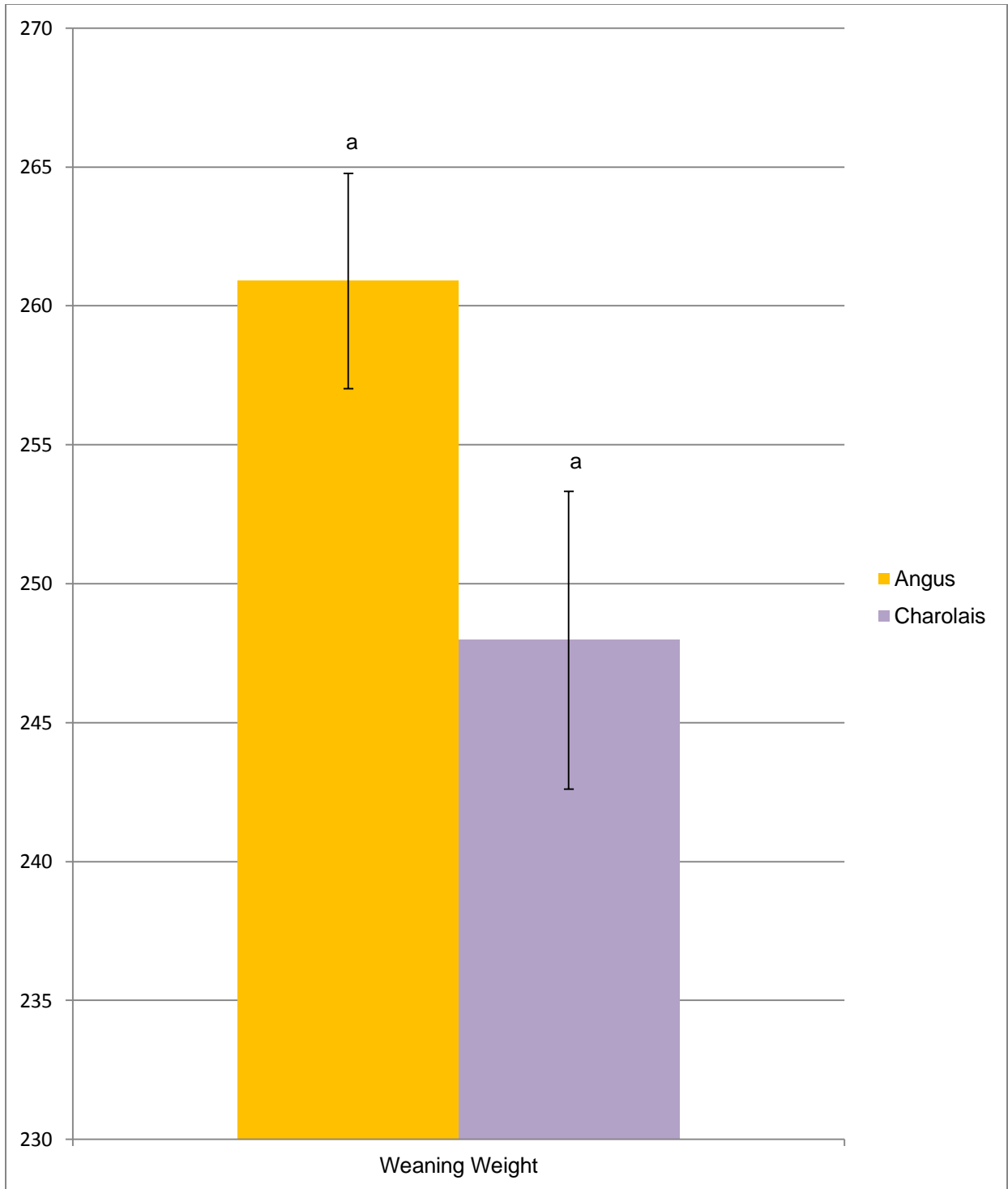


Figure 3.2: Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of weaning weight.

^a Superscript indicates no significance at $p < 0.05$

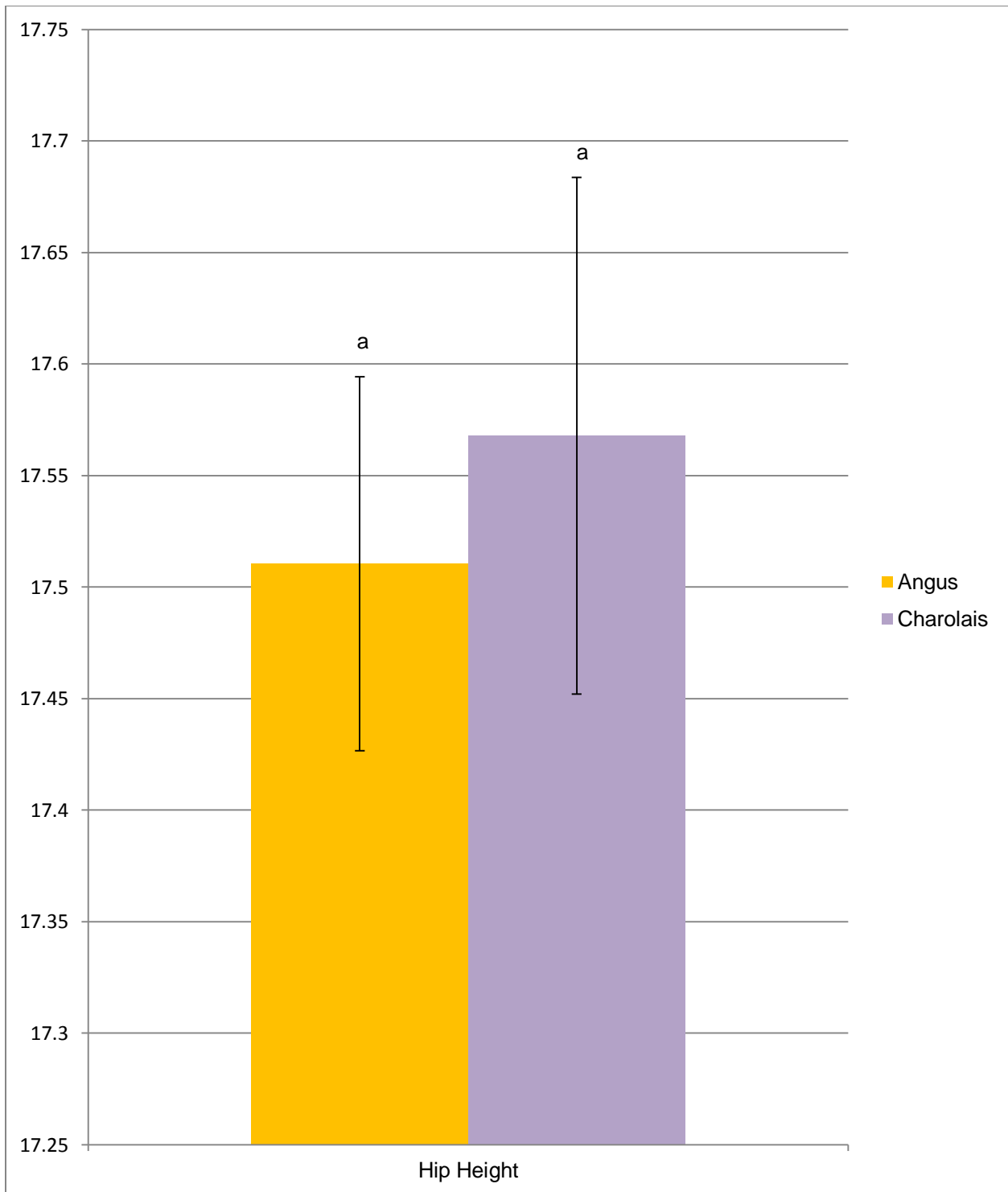


Figure 3.3: Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of hip height.

^a Superscript indicates no significance at $p < 0.05$

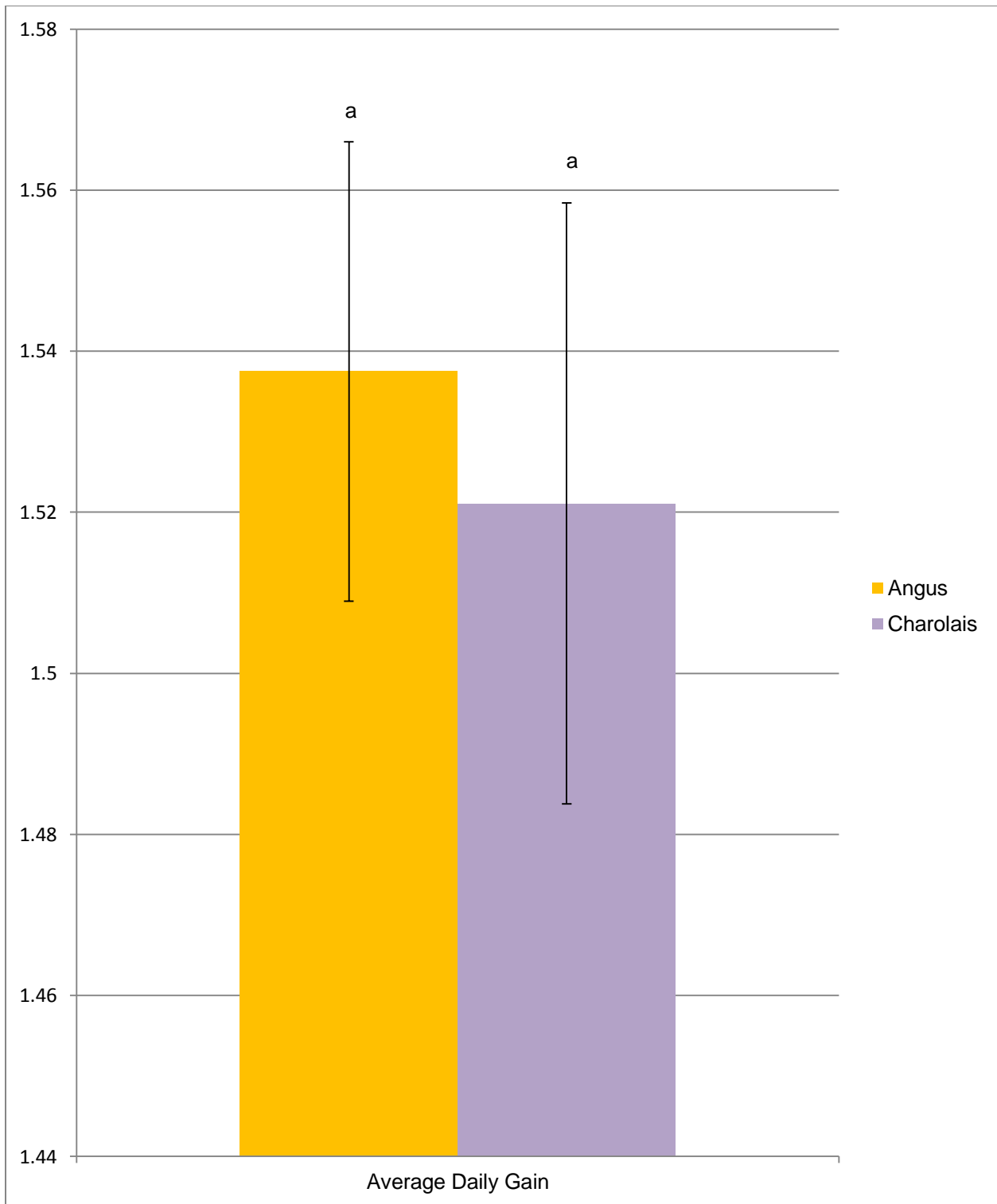


Figure 3.4: Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of average daily gain in the feedlot.

^a Superscript indicates no significance at $p < 0.05$

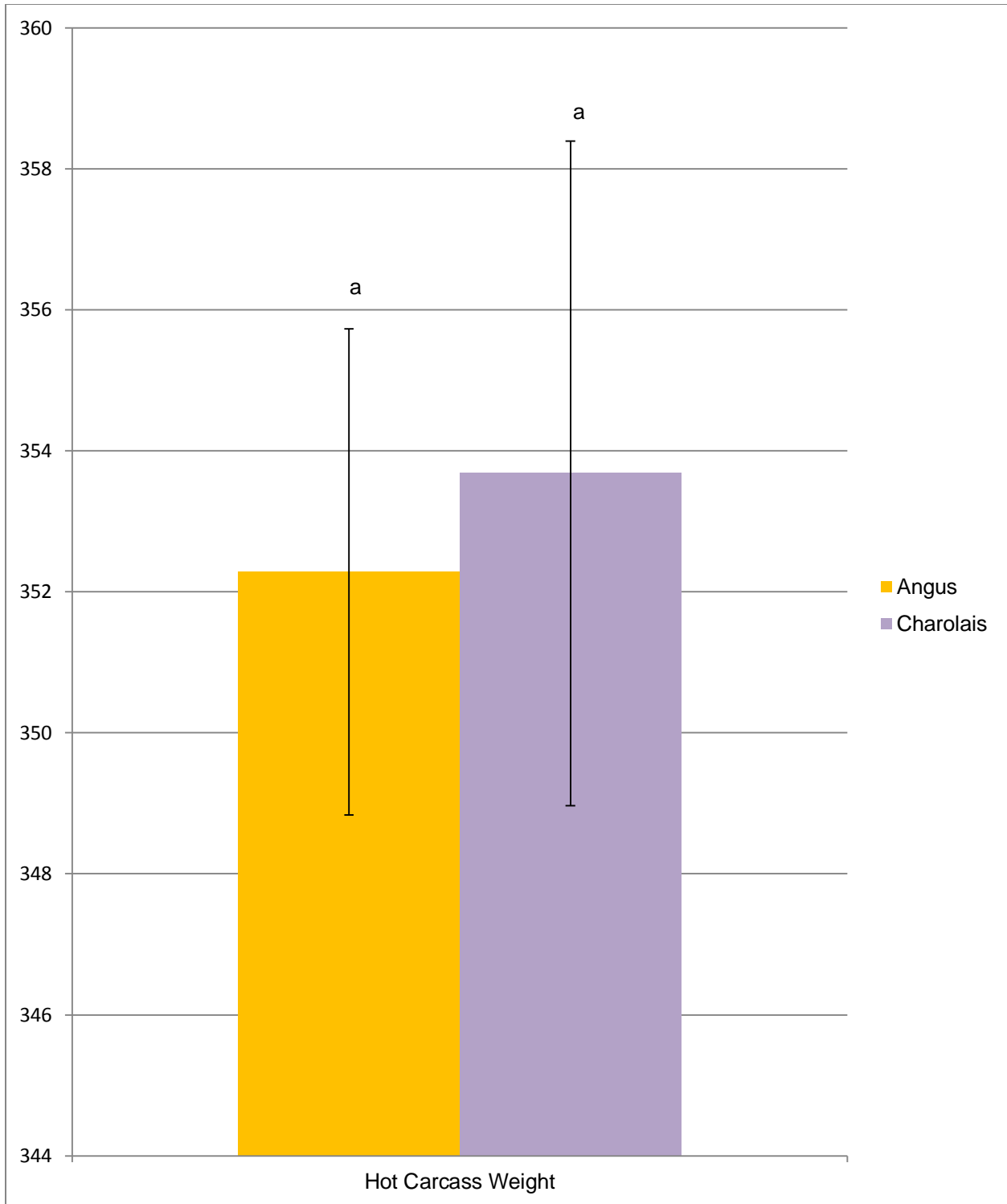


Figure 3.5: Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of hot carcass weight.

^a Superscript indicates no significance at $p < 0.05$

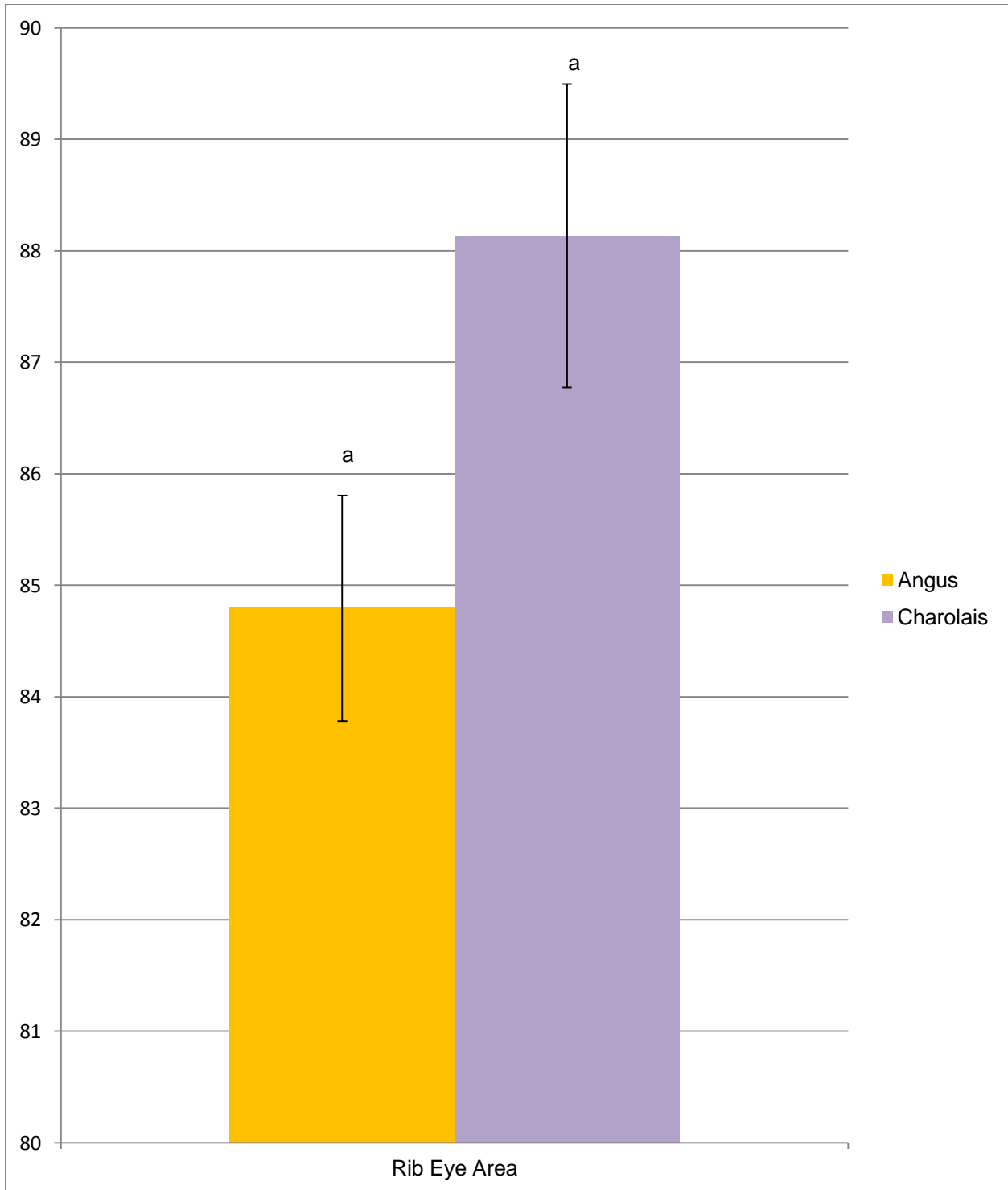


Figure 3.6: Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of rib eye area.

^a Superscript indicates no significance at $p < 0.05$

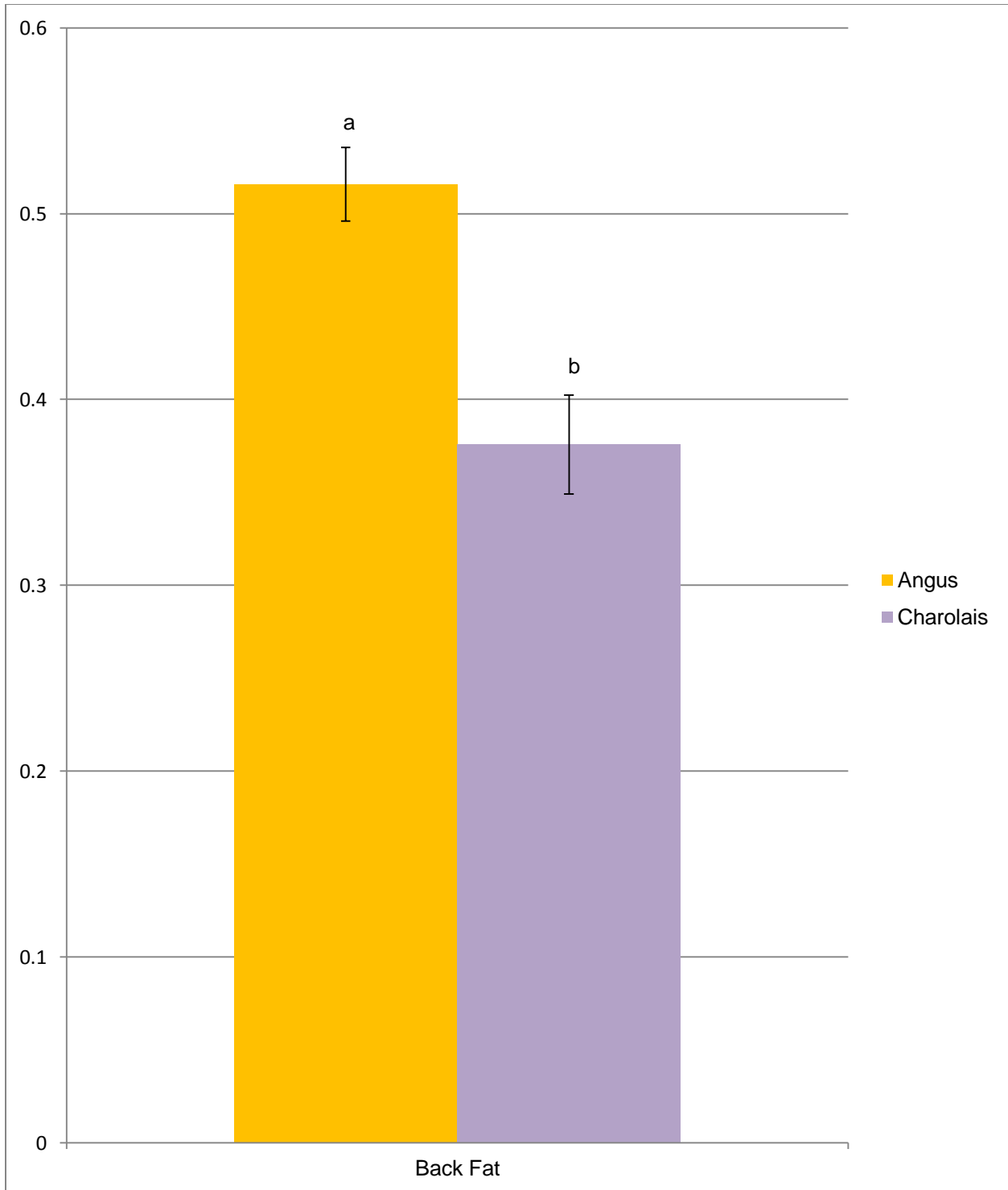


Figure 3.7: Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of back fat.

^{a, b} Superscript indicates significance at $p < 0.05$

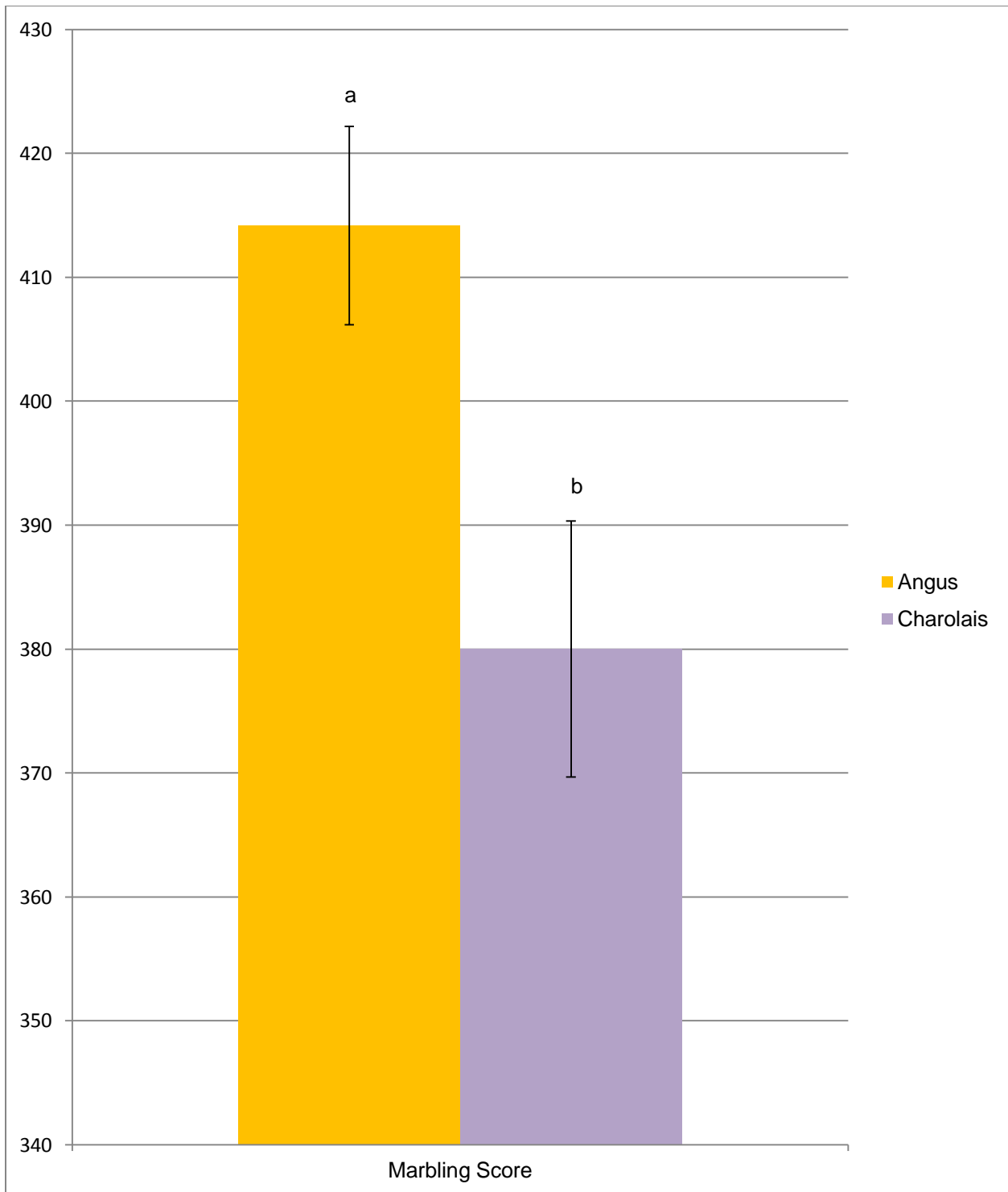


Figure 3.8: Means separation analysis comparing multigenerational Angus sired calves and Charolais sired calves for the trait of marbling score.

^{a, b} Superscript indicates significance at $p < 0.05$

Charolais sired animals and were validated by Gregory and associates (1994), DeRouen and associates (1992), and Williams and associates (2012). The results of the current study indicate that multigenerational Angus sired animals had significantly larger back fat thickness measurements than Charolais sired animals. Studies conducted by Williams and associates (2010) and DeRouen and associates (1992) reported an association between Angus sired animals and larger back fat thickness measurements when compared to Charolais sired animals.

The implications of this study are the potential benefits of incorporating multigenerational animals into current production schemes in order to improve traits which have been inadvertently selected against. Incorporation of such genetics could improve traits such as fertility without hindering improvements made on other economically important traits. Integrating multigenerational genetics also has the potential benefit of improving carcass quality traits without decreasing carcass size. The differences observed between the two sire breed types for carcass quality and composition traits were as expected.

CHAPTER IV
AN SNP ASSOCIATION STUDY EVALUATING MODERN CHAROLAIS SIRE
CALVES VERSUS MULTIGENERATIONAL ANGUS SIRE CALVES FOR GROWTH
AND CARCASS TRAITS

Introduction

Identification of molecular markers associated with economically important traits has the capability of greatly improving the accuracy and rate of improvement for these traits. Single nucleotide polymorphisms are a type of molecular marker that can be evaluated for improvement of economically important traits due to genetic variation. Utilization of SNP's on candidate genes may provide insight into molecular variant's contribution to variation observed in economically important traits.

In the current study, the calpain III (CAPN3) and calpastatin (CAST) genes were selected as candidate genes for growth and carcass traits due to previous reports of their involvement with carcass quality and composition traits (Illian et al., 2001; Barendse et al., 2008). The calpain III gene has been reported to have an effect on growth and meat tenderness (Spencer et al., 2002; Barendse et al., 2008). The calpastatin gene has been previously described as having an association with protein degradation and meat tenderness (Killefer and Koohmaraie, 1994) and as such may have a role in growth. A previous study conducted by Schenkel and associates (2006) reported an SNP on calpastatin to be associated with rib eye area.

The objective of this study is to evaluate the association of single nucleotide polymorphisms in the CAST and CAPN3 genes for growth, performance, and carcass quality and composition characteristics in a population consisting of multigenerational Angus sired calves and modern Charolais sired calves.

Experimental Animals

The population utilized in the current study consisted of one hundred thirty-two animals sired by purebred multigenerational Angus or modern Charolais sires mated to crossbred dams. The dam lines were comprised of one of four crossbred lines from the Germplasm Evaluation VIII population described in previous studies (Wheeler et al., 2005). The Angus sires utilized in the current study consisted of multigenerational sires born between 1960 and 2006. Dams had estrous synchronized via a previously described procedure (Wilson et al., 2010) and multigenerational Angus bulls were mated to the GPE VIII female population via artificial insemination. Upon completion of artificial insemination, Charolais sires were utilized as cleanup bulls for 67 days to increase pregnancy rates in the GPE VIII cows. The Charolais bulls were modern in their phenotype and contained modern EPD's typically utilized for a terminal herd sire.

All calves were born between January 15 and April 23, 2010 at the LSU AgCenter Central Research Station in Baton Rouge, LA. Calves were managed at this facility until weaning, or approximately six months of age. The population consists of 86 multigenerational Angus sired animals and 46 Charolais sired animals. Animals were nominated for the Louisiana calf to carcass program after a 45 day preconditioning program and vaccination program that was established by the Louisiana calf to carcass program. Animals were then sent to either Hitch feedlot or Buffalo feedlot until a final harvest weight was obtained.

Growth and Carcass Characteristics

Growth and performance traits collected at the Central Research Stations in Baton Rouge, LA included birth weight (BW), weaning weight (WW), and hip height

(HH). However, upon leaving the research station, data collected by the calf to carcass program included weight post shipping (shrinkage), days on feed, finishing weight and average daily gain (ADG). Animals were finished at either Hitch feedlot or Buffalo feedlot until a desired harvest weight of over 1200 lbs. was reached. All animals were then transported to a commercial packing plant for harvest and subsequent carcass quality and composition trait measurement recording. Carcass quality and composition measurements that were collected included hot carcass weight (HCW), yield grade (YG), rib eye area (REA), back fat thickness (BF), marbling score (MARB) and quality grade (QG).

Candidate Gene Selection

Two candidate genes were selected for the current study based on previously reported associations with traits evaluated in this study. The first candidate gene evaluated was the calpain III (CAPN3) gene found on BTA 10. The calpain III gene spans from 37,829K to 37885K base pairs on BTA 10 (www.ncbi.nlm.nih.gov). The CAPN3 gene is a skeletal muscle specific calpain and is inferred to play a role in skeletal muscle differentiation and development (Spencer et al., 2002) and on meat tenderness (Ilian et al., 2001). Previous studies observed significant associations between SNP at the CAPN3 gene on chromosome 10 and meat tenderness (Barendse et al., 2008; Robinson et al., 2012).

The second candidate gene evaluated was the calpastatin (CAST) gene located on BTA 7. The CAST gene spans from 98,444K to 98,581K base pairs on BTA 7 (www.ncbi.nlm.nih.gov). The CAST gene is associated with the production of calpastatin which is a specific inhibitor of calpains. Multiple studies have observed calpastatin's

association with meat tenderness, fat yield, and rib eye area (Killefer and Koohmaraie, 1994; Huang and Forsberg, 1998; Ilian et al., 2001; Schenkel et al., 2006).

SNP Selection

Previously reported single nucleotide polymorphisms (SNP) on CAPN3 and CAST were selected equidistant across each of the genes from the dbSNP database available at <http://www.ncbi.nlm.nih.gov/gene/281039> in order to evaluate possible linkage associations with potential causative mutations. Twenty SNP were selected for both the CAST gene and the CAPN3 gene. The Sequenom genotyping platform (Illumina Inc., San Diego, California) was used to visualize individual genotypes for individual SNP's for every animal and was performed by NeoGen (Lincoln, Nebraska).

DNA Extraction

Twenty mL of blood was extracted via jugular venipuncture from every animal on study and stored at 4° C until transferred into 20mL tubes and centrifuged at 4000 rpm for 30 minutes. White blood cell buffy coats were extracted and DNA was isolated and purified from buffy coats using a Saturated Salt procedure as previously described by Miller and associates (1988) (Appendix A). Working solutions were prepared for genotyping by diluting the purified DNA to a 250ng/ μ L concentration necessary for genotyping.

Statistical Analysis

The analyses conducted in this study were performed using the mixed procedure of SAS as previously described by White and associates (2005). Fixed effects include sire breed, sex and marker genotype. Random effects included the birth weight, weaning weight, hip height, average daily gain, hot carcass weight, rib eye area, back

fat thickness, and marbling score. The LSMEANS and pre-planned pairwise comparisons functions of SAS were utilized for means separation analysis based on inheritance patterns of SNP genotype. The means and marker effect of the birth weight, weaning weight, hip height, hot carcass weight, average daily gain, back fat thickness, rib eye area, and marbling score were compared between the multigenerational Angus sired calves and the Charolais sired calves. Due to a limited sample population statistical significance was set at $p < 0.05$ and statistical trends were set at $p < 0.1$.

Results

SNP ASSOCIATED WITH WEANING WEIGHT

Two SNPs located on the CAPN3 gene were shown to be significantly ($p < 0.05$) associated with weaning weight and one SNP showed a trend ($p < 0.1$) towards weaning weight. The first SNP significantly ($p < 0.05$) associated with weaning weight was rs109806627 (Table 4.3). Animals inheriting the heterozygous genotype GC had significantly heavier ($p < 0.05$) weaning weights than animals that inherited the homozygous G genotype (Table 4.4). The second SNP significantly ($p < 0.05$) associated with weaning weight was rs136324366 (Table 4.3). Animals inheriting the homozygous C genotype for this SNP had significantly heavier ($p < 0.05$) weaning weights than animals inheriting the heterozygous CA genotype (Table 4.4). A trend ($p < 0.1$) towards weaning weight was observed for rs109337751 located on CAPN3 (Table 4.3). Animals with the heterozygous CT genotype had significantly ($p < 0.05$) heavier weaning weights than animals inheriting the homozygous T genotype (Table 4.4).

Table 4.1: SNP ID, forward and reverse primer sequences and allele substitution at SNP location utilized to obtain genotypes for 20 SNP located on the CAPN3 gene.

CAPN3 SNPs			
Name	Allele substitution	Forward Primer Sequence	Reverse Primer Sequence
rs135512997	A/G	TCAGGGTGCCATGGGATCAGAAGGCA	GGAGAGTTAACCTAGGCCAGAGAGA
rs43624105	C/G	GACTCCCACCTGCCTTACACATTTGT	TCATTTATCCATTGGTTCAAACTC
rs109372443	A/G	TTATCCAAATGTGCTTTTATATGTCA	TCCTCAGCATGGTGGAAATCTTCAGC
rs109050259	A/G	TAAGTCAGATGGATTTAGAAGCAAAC	GACCTGGTCGGAACCCTGACTCTGT
rs110822150	G/T	CCTCCTGATGGACAGGCTGCCAGAAT	GAGTTTCTCCCCGGGCCAGCTCCTT
rs135091523	G/T	TTCTCAACATTAATATGATGATAAT	GTAGGTCTCTAAAAAAGGTACATT
rs110452450	A/G	TGCTAGGTTGATGGGTTTTTGTATCA	TTTAAATATTTCAATCCGATCTTTC
rs133798263	C/T	AGGACGCCTGCGGCCTTCGTAGGTGA	GTAATTCCCTGCTTTGGAATTTA
rs41644730	C/T	CCTTGGGTCTACAGGTTCAGAAGCCT	GTCCCCCATCTGCCACCAATTCCA
rs134606436	A/C	GAACAGCCACGTCATCAGTGCATGCG	AGTCACGTGATCAGTGCATGAGAGC
rs109337751	C/T	GGGTGGTGTGTGTGGTGTGATCTATG	ACGACTTTCATCTAGTAGGTGAGA
rs109806627	C/G	CCTCCCCTCTGCAGTCTCAAAGATGC	GTGACATCCCACACGGCACAGCAA
rs110247569	C/G	ACTGGAAGACTTACCCAGTGAACAG	AGCACAGGGCTTTCCTCTGGGGCAG
rs137379223	A/G	TGCTCAGTTTATGTTTATTTTAGTG	TAAAGATAGCAATTGCCCAACATT
rs134085397	A/G	GCTATATTCTCCAGTCTCAGATCTTG	GGTCCACTTTCTGCCAACTCCTCCA
rs136966673	A/G	GACATACAGATGGCTAACAAACACAT	AAAAGATGCTCAACATCACTCATCA
rs109122904	A/G	TGCAGCGTTACCAGGGTCTGGGTCC	CCTGGGGCACATCAGGGCACCTCCT
rs43624108	G/T	TTTATAGTCCATTCTGCCATTGGTCG	GGCCAAGGAAGGGGAGCTTCTGGCA
rs43624107	A/C	ACACAACCTGGGGCCCAAGCATGGGT	TGGGCCACACAGGCCATGGAGTGTA
rs136324366	A/C	TCTGGTCTGCTTCATCCTGCTGGGG	TGGTTTGGTTGGGATGGTCAGGCTG

Table 4.2: SNP ID, forward and reverse primer sequences and allele substitution at SNP location utilized to obtain genotypes for 20 SNP located on the CAST gene.

CAST SNPs			
Name	Allele substitution	Forward Primer Sequence	Reverse Primer Sequence
rs137382340	C/T	ACTTTTTTAAAGAATGACGTAGCTTGC	GCTGGTGGTATAAATGGTGGGTGGC
rs109236372	A/G	TTAGAAAATTTTTATAATAAAAAGCC	GGTTAAAAAACTAGAAAAATATA
rs110386026	C/T	AAGCCAGGCCTTTGAAATAAAGTCAG	TTGAAAATGTGCTTTCTCTGCTCAG
rs133120980	A/C	GGAAGGAGCACCTAGGCTGAGGATAG	CCCGGGACTGGGAGCTGAGGGAGCT
rs109594429	C/G	GCGGATCTTGATGACGCTGGATCACC	TCCCACACACACACTCACACACCGT
rs110374623	C/T	TAGTCTGAATTTTGCTTGAAAAGATA	TTGTATTGACAGAAAAATTTGCGGT
rs109813625	A/T	CTCTTTACCGTCTGAGCCACCAGGGA	GACAAGGCTAGATATGGTTAAAATG
rs134030456	C/T	CCAGTGGAGTTACTCTGATCCCCCGC	GCACACTTTTCCGTATGCTGTTATG
rs110145113	C/G	ATGAACACACCCTCATTAAATCGCTCA	TACCATCTTATGAGGTAGACGTCAC
rs110458074	A/G	GGGAGATCAAACCAGTCAATTCCAAA	GAAATCAATCCTAAATAGTCATTGG
rs133891017	A/T	AATCAAGCAGAAACTAAAGATCAGAG	GTTTAAGTAGCTTGCCCAAGATTAT
rs135521006	C/T	GGAAGGAAAGTTATGATCAGCCTAGA	AGCATATTCAAAGCAGAGACATTA
rs134520907	-/AT	GCTTGTGAGGTCTGTTTATGAGAAAC	ATGCTCAGCGAAGACCCCTTCTCCC
rs109906719	A/G	TTATGTAACACTTGAAAATAGGCAAA	TAATGGTGTATATTGTTTATGGATA
rs110136749	A/G	GAAATTAGAAATCTGTCTTTGCAAAA	GGTTCTAGGTGCTTCTATTTATAAA
rs137371179	C/T	ACTCACTCAAACATATCAGAAAAAGA	AGTCTCTTCTCATATCCTGGAAT
rs109020860	A/G	CCTGTCGTCCTGAGTGTGACTGCCAG	GCTTAAGAGCAAGTTAGCTCCTTTC
rs109702795	G/T	CCATCAGGAAATAYGGTCCAGCACAC	GGATGTTTTCAACAGTGTAACATAAT
rs109572153	A/C	CAAATAAGACATAAAAACACAAGTTA	CTTTTGTGATGTTTTAACTAGAGTT
rs110496242	A/G	ACCTTTGGTAGATAAACGAAAGAGAA	GGAACCTACTGTAGGAAATCATCAA

SNP ASSOCIATED WITH HIP HEIGHT

Three SNPs located on the calpain III gene were shown to be significantly ($p < 0.05$) associated with hip height. The first SNP significantly associated with hip height was rs109337751 (Table 4.3). Animals inheriting the heterozygous CT genotype had significantly higher ($p < 0.05$) hip height measurements than animals inheriting the homozygous C genotype (Table 4.4). The second SNP significantly associated with hip height was rs109806627 (Table 4.3). Animals inheriting the heterozygous GC genotype had significantly higher ($p < 0.05$) hip height measurements than animals inheriting the homozygous C genotype for this SNP (Table 4.4). Single nucleotide polymorphism rs110822150 was also significantly associated with hip height (Table 4.3). Animals inheriting the heterozygous GT genotype for this SNP had significantly higher ($p < 0.05$) hip height measurements than animals inheriting the homozygous G genotype (Table 4.4).

Table 4.3: Level of significance, numbers of animals from each genotype, and S.E. for markers significantly associated with weaning weight and hip height.

Trait	Gene	SNP ID	Allele ³	Minor Genotype Frequency ⁴	Heterozygous Frequency ⁴	Major Genotype Frequency ⁴	P-value
WW ¹	CAPN3	rs109337751	T/C	7	52	67	0.0718
WW	CAPN3	rs109806627	G/C	7	53	63	0.0392
WW	CAPN3	rs136324366	A/C		1	126	0.0344
HH ²	CAPN3	rs109337751	T/C	7	52	67	0.0071
HH	CAPN3	rs109806627	G/C	7	53	63	0.0042
HH	CAPN3	rs110822150	T/G	8	50	61	0.0081

¹Weaning Weight

²Hip Height

³Representation of minor allele is located on the left

⁴ Number of animals inheriting each genotype

Table 4.4: Single nucleotide polymorphisms significantly associated with weaning weight and hip height and least square means comparisons between reported genotypes.

Trait	Gene	SNP ID	Allele ³	Minor Genotype Mean	Heterozygous Mean	Major Genotype Mean
WW ¹	CAPN3	rs109337751	T/C	231.09 ± 13.7744 ^a	260.58 ± 5.4154 ^b	250.49 ± 4.3304 ^{ab}
WW	CAPN3	rs109806627	G/C	231.38 ± 13.6346 ^a	261.27 ± 5.2756 ^b	248.41 ± 4.4205 ^{ab}
WW	CAPN3	rs136324366	A/C		178.48 ± 35.0881 ^a	253.67 ± 3.2887 ^b
HH ²	CAPN3	rs109337751	T/C	111.72 ± 1.8852 ^{ab}	114.89 ± 0.7412 ^a	112.03 ± 0.5926 ^b
HH	CAPN3	rs109806627	G/C	111.71 ± 1.8875 ^{ab}	114.96 ± 0.7303 ^a	111.98 ± 0.6119 ^b
HH	CAPN3	rs110822150	T/G	112.02 ± 1.7648 ^{ab}	115.05 ± 0.7617 ^a	112.03 ± 0.6297 ^b

¹Weaning Weight (kg)

²Hip Height (cm)

³Representation of minor allele is located on the left

^{a,b} Superscripts indicate difference within row

SNP ASSOCIATED WITH AVERAGE DAILY GAIN

Only one SNP was significantly ($p < 0.05$) associated with average daily gain, and three SNP showed a trend ($p < 0.1$) towards average daily gain. On CAPN3, SNP rs109122904 (Table 4.5) was significantly associated with average daily gain with animals inheriting the heterozygous GA genotype having significantly higher ($p < 0.05$) average daily gains than animals that inherited the homozygous G genotype (Table 4.6). The first SNP showing a trend ($p < 0.1$) towards average daily gain was rs109372443 (Table 4.5). Animals inheriting the homozygous G genotype had significantly higher ($p < 0.05$) average daily gains than animals inheriting the homozygous A genotype (Table 4.6). The second SNP that exhibited a trend was rs135091523 (Table 4.5). The third SNP that showed a trend ($p < 0.1$) towards average daily gain was rs135512997 (Table 4.5).

Table 4.5: Level of significance, numbers of animals from each genotype, and S.E. for markers significantly associated with average daily gain.

Trait	Gene	SNP ID	Allele ²	Minor Genotype Frequency ³	Heterozygous Frequency ³	Major Genotype Frequency ³	P-value
ADG ¹	CAPN3	rs109122904	A/G	1	32	95	0.0205
ADG	CAPN3	rs109372443	G/A	17	63	44	0.0840
ADG	CAPN3	rs135091523	G/T		11	113	0.0848
ADG	CAPN3	rs135512997	G/A		11	116	0.0962

¹Average Daily Gain

²Representation of minor allele is located on the left

³Number of animals inheriting each genotype

Table 4.6: Single nucleotide polymorphisms significantly associated with average daily gain and least square means comparisons between reported genotypes.

Trait	Gene	SNP ID	Allele ²	Minor Genotype Mean	Heterozygous Mean	Major Genotype Mean
ADG ¹	CAPN3	rs109122904	A/G	1.8411 ± 0.2105 ^{ab}	1.6135 ± 0.0467 ^a	1.4882 ± 0.0257 ^b
ADG	CAPN3	rs109372443	G/A	1.6228 ± 0.06430 ^a	1.5318 ± 0.03244 ^{ab}	1.4593 ± 0.03926 ^b

¹Average Daily Gain (kg)

²Representation of minor allele is located on the left

^{a,b} Superscripts indicate difference within row

SNP ASSOCIATED WITH HOT CARCASS WEIGHT

Three SNP located on CAPN3 were significantly ($p < 0.05$) associated with hot carcass weight and one SNP showed a trend ($p < 0.1$) for hot carcass weight. Single nucleotide polymorphism rs109337751 was significantly associated with hot carcass weight (Table 4.7). Animals inheriting the homozygous T genotype for this SNP had significantly ($p < 0.05$) lower hot carcass weights than animals inheriting the homozygous C genotype and significantly ($p < 0.05$) lower hot carcass weights than animals inheriting the heterozygous CT genotype for this SNP (Table 4.8).

The second SNP located on CAPN3 significantly associated with hot carcass weight was rs109806627 (Table 4.7). Animals inheriting the homozygous C genotype

had significantly greater ($p < 0.05$) hot carcass weights than animals inheriting the homozygous G genotype. Animals inheriting the heterozygous GC genotype also had significantly greater ($p < 0.05$) hot carcass weights than animals inheriting the homozygous G genotype. No significant difference was observed between animals inheriting the homozygous C genotype and animals inheriting the heterozygous GC genotype (Table 4.8).

The third SNP on CAPN3 significantly associated with hot carcass weight was rs110822150 (Table 4.7). Animals inheriting the homozygous T genotype for this SNP had significantly ($p < 0.05$) lower hot carcass weights than animals inheriting the homozygous G genotype. Animals inheriting the homozygous T genotype also had significantly ($p < 0.05$) lower hot carcass weights than animals inheriting the heterozygous GT genotype. No significant difference was observed between animals inheriting the heterozygous GT genotype and animals inheriting the homozygous G genotype (Table 4.8).

Table 4.7: Level of significance, numbers of animals from each genotype, and S.E. for markers significantly associated with hot carcass weight.

Trait	Gene	SNP ID	Allele ²	Minor Genotype Frequency ³	Heterozygous Frequency ³	Major Genotype Frequency ³	P-value
HCW ¹	CAPN3	rs109337751	T/C	7	52	67	0.0129
HCW	CAPN3	rs109806627	G/C	7	53	63	0.0166
HCW	CAPN3	rs110822150	T/G	8	50	61	0.0382
HCW	CAPN3	rs41644730	T/C	12	5	109	0.0638

¹Hot Carcass Weight

²Representation of minor allele is located on the left

³Number of animals inheriting each genotype

A trend ($p < 0.1$) was observed between SNP rs41644730 and hot carcass weight (Table 4.7). Animals inheriting the homozygous C genotype had significantly greater ($p < 0.05$) hot carcass weights than animals inheriting the homozygous T genotype (Table 4.8).

Table 4.8: Single nucleotide polymorphisms significantly associated with hot carcass weight and least square means comparisons between reported genotypes.

Trait	Gene	SNP ID	Allele ²	Minor Genotype Mean	Heterozygous Mean	Major Genotype Mean
HCW ¹	CAPN3	rs109337751	T/C	319.08 ± 12.2081 ^a	356.90 ± 4.7967 ^b	353.93 ± 3.8109 ^b
HCW	CAPN3	rs109806627	G/C	319.30 ± 12.0535 ^a	355.33 ± 4.6611 ^b	353.51 ± 3.8784 ^b
HCW	CAPN3	rs110822150	T/G	325.12 ± 11.1940 ^a	355.38 ± 4.8276 ^b	353.51 ± 3.9631 ^b
HCW	CAPN3	rs41644730	T/C	331.12 ± 9.3939 ^a	351.46 ± 14.0473 ^{ab}	354.14 ± 3.0953 ^b

¹Hot Carcass Weight (kg)

²Representation of minor allele is located on the left

^{a,b} Superscripts indicate difference within row

SNP ASSOCIATED WITH MARBLING SCORE

One SNP located on the CAPN3 gene was significantly ($p < 0.05$) associated with marbling score and two SNP showed a trend ($p < 0.1$) towards marbling score (Table 4.9). The SNP showing a significant association with marbling score was rs41644730 and animals inheriting the homozygous T genotype for this SNP had significantly ($p < 0.05$) higher marbling scores than animals inheriting the homozygous C genotype (Table 4.10). Single nucleotide polymorphism rs109050259 exhibited a trend ($p < 0.1$) towards marbling score (Table 4.9). Animals inheriting the homozygous A genotype had significantly ($p < 0.05$) higher marbling scores than animals inheriting the heterozygous AG genotype and had significantly ($p < 0.05$) higher marbling scores

than animals inheriting the homozygous G genotype (Table 4.10). The second SNP showing a trend ($p < 0.1$) towards marbling score was rs109372443 (Table 4.9). Animals inheriting the homozygous A genotype had significantly higher ($p < 0.05$) marbling scores than animals inheriting the heterozygous AG genotype for this SNP (Table 4.10).

Table 4.9: Level of significance, numbers of animals from each genotype, and S.E. for markers significantly associated with marbling score.

Trait	Gene	SNP ID	Allele ²	Minor Genotype Frequency ³	Heterozygous Frequency ³	Major Genotype Frequency ³	P-value
MARB ¹	CAPN3	rs109050259	G/A	14	65	46	0.0588
MARB	CAPN3	rs109372443	G/A	17	63	44	0.0516
MARB	CAPN3	rs41644730	T/C	12	5	109	0.0219

¹Marbling Score

²Representation of minor allele is located on the left

³Number of animals inheriting each genotype

Table 4.10: Single nucleotide polymorphisms significantly associated with marbling score and least square means comparisons between reported genotypes.

Trait	Gene	SNP ID	Allele ²	Minor Genotype Mean	Heterozygous Mean	Major Genotype Mean
MARB ¹	CAPN3	rs109050259	G/A	372.27 ± 18.0498 ^a	388.46 ± 8.9328 ^a	416.37 ± 11.2272 ^b
MARB	CAPN3	rs109372443	G/A	378.58 ± 17.3195 ^{ab}	385.85 ± 8.9383 ^a	417.89 ± 11.4053 ^b
MARB	CAPN3	rs41644730	T/C	443.35 ± 21.5762 ^a	436.23 ± 32.3405 ^{ab}	387.77 ± 6.3412 ^b

¹Marbling Score

²Representation of minor allele is located on the left

^{a,b} Superscripts indicate difference within row

SNP ASSOCIATED WITH RIB EYE AREA

One SNP located on the CAST gene as significantly ($p < 0.05$) associated with rib eye area and two SNP on the CAPN3 gene showed a trend ($p < 0.1$) towards rib eye area. The SNP on the CAST gene significantly ($p < 0.05$) associated with rib eye area was rs109906719 (Table 4.11). Animals inheriting the homozygous G genotype had significantly larger ($p < 0.05$) rib eye area than animals inheriting the homozygous A genotype (Table 4.12). The first SNP on the CAPN3 gene to exhibit a trend ($p < 0.1$) for rib eye area was rs109337751 (Table 4.11). Animals inheriting the homozygous T genotype had significantly smaller ($p < 0.05$) rib eye areas than animals inheriting the heterozygous CT genotype (Table 4.12). The second SNP that showed a trend was rs109806627 (Table 4.11). Animals inheriting the homozygous G genotype had significantly smaller ($p < 0.05$) rib eye areas than animals inheriting the heterozygous GC genotype (Table 4.12).

Table 4.11: Level of significance, numbers of animals from each genotype, and S.E. for markers significantly associated with rib eye area.

Trait	Gene	SNP ID	Allele ²	Minor Genotype Frequency ³	Heterozygous Frequency ³	Major Genotype Frequency ³	P-value
REA ¹	CAST	rs109906719	G/A	1		101	0.0002
REA	CAPN3	rs109337751	T/C	7	52	67	0.0887
REA	CAPN3	rs109806627	G/C	7	53	63	0.0986

¹Rib eye area

²Representation of minor allele is located on the left

³Number of animals inheriting each genotype

Table 4.12: Single nucleotide polymorphisms significantly associated with rib eye area and least square means comparisons between reported genotypes.

Trait	Gene	SNP ID	Allele ²	Minor Genotype Mean	Heterozygous Mean	Major Genotype Mean
REA ¹	CAST	rs109906719	G/A	120.76 ± 8.691 ^a		86.10 ± 0.9619 ^b
REA	CAPN3	rs109337751	T/C	78.431 ± 3.897 ^a	87.312 ± 1.4252 ^b	86.208 ± 1.140 ^{ab}
REA	CAPN3	rs109806627	G/C	78.702 ± 3.845	87.228 ± 1.392	85.968 ± 1.151

¹Rib eye area (cm²)

²Representation of minor allele is located on the left

^{a,b} Superscripts indicate difference within row

SNP ASSOCIATED WITH BACK FAT THICKNESS

Four SNP were significantly ($p < 0.05$) associated with back fat thickness. The first SNP significantly associated with back fat thickness was rs109020860 located on the CAST gene (Table 4.13). Animals inheriting the homozygous A genotype had significantly ($p < 0.05$) larger back fat thickness measurements than animals inheriting the homozygous G genotype. Animals inheriting the homozygous A genotype also had significantly ($p < 0.05$) larger measurements than animals inheriting the heterozygous GA genotype. Animals inheriting the homozygous G genotype had significantly ($p < 0.05$) larger back fat thickness measurements than animals inheriting the heterozygous GA genotype (Table 4.14).

The second SNP significantly associated with back fat thickness was rs134085397 located on the CAPN3 gene (Table 4.13). Animals inheriting the homozygous A genotype for this SNP had significantly ($p < 0.05$) larger back fat thickness measurements than animals inheriting the homozygous G genotype for this SNP (Table 4.14).

The third SNP significantly associated with back fat thickness was rs135091523 located on the CAPN3 gene (Table 4.13). Animals inheriting the homozygous T

genotype had significantly ($p < 0.05$) larger back fat thickness measurements than animals inheriting the heterozygous GT genotype (Table 4.14). The last SNP significantly associated with back fat thickness was rs135512997 (Table 4.13). Animals inheriting the homozygous A genotype had significantly ($p < 0.05$) larger back fat thickness measurements than animals inheriting the heterozygous GA genotype (Table 4.14).

Table 4.13: Level of significance, numbers of animals from each genotype, and S.E. for markers significantly associated with back fat.

Trait	Gene	SNP ID	Allele ²	Minor Genotype Frequency ³	Heterozygous Frequency ³	Major Genotype Frequency ³	P-value
BF ¹	CAST	rs109020860	A/G	1	18	108	0.0098
BF	CAPN3	rs 134085397	A/G	22	73	30	0.0281
BF	CAPN3	rs135091523	G/T		11	113	0.0103
BF	CAPN3	rs 135512997	G/A		11	116	0.0142

¹Back fat

²Representation of minor allele is located on the left

³Number of animals inheriting each genotype

Table 4.14: Single nucleotide polymorphisms significantly associated with back fat and least square means comparisons between reported genotypes.

Trait	Gene	SNP ID	Allele ²	Minor Genotype Mean	Heterozygous Mean	Major Genotype Mean
BF ¹	CAST	rs109020860	A/G	2.1582 ± 0.4516	0.9256 ± 0.1064	1.1692 ± 0.0454 ^c
BF	CAPN3	rs 134085397	A/G	1.3353 ± 0.0967 ^a	1.1326 ± 0.0563 ^{ab}	0.9865 ± 0.0852 ^b
BF	CAPN3	rs135091523	G/T		0.8176 ± 0.1354 ^a	1.1930 ± 0.0451 ^b
BF	CAPN3	rs 135512997	G/A		0.8179 ± 0.1372 ^a	1.1803 ± 0.0451 ^b

¹Back fat (cm)

²Representation of minor allele is located on the left

^{a,b,c} Superscripts indicate difference within row

Discussion

Significant associations were observed between CAPN3 SNP rs109337751 and the traits hip height and hot carcass weight. A trend was observed between rs109337751 and weaning weight and rib eye area. CAPN3 SNP rs109806627 was significantly associated with weaning weight, hip height, and hot carcass weight, but showed an association without significance for rib eye area. A significant association was observed between CAPN3 SNP rs136324366 and weaning weight. Significant associations were observed between CAPN3 SNP rs110822150 and the traits hip height and hot carcass weight. This is the first study to report significant associations between these SNP and the traits of weaning weight, hip height, and hot carcass weight.

A trend was observed between CAPN3 SNP rs109372443, average daily gain, and marbling score. The SNP rs109122904 on CAPN3 showed a significant association with average daily gain. A trend was observed between CAPN3 SNP rs135091523 and average daily gain, and a significant association was observed between this SNP and back fat thickness. A trend was also observed between CAPN3 SNP rs135512997 and average daily gain, and a significant association was observed between this SNP and back fat thickness. This is the first study to report significant associations between these SNP and the traits average daily gain, marbling score, and back fat thickness.

The CAPN3 SNP rs41644730 showed an association without significance with hot carcass weight, but showed a significant association with marbling score. A trend was observed between CAPN3 SNP 109050259 and marbling score. A significant association was observed between the CAPN3 SNP rs134085397 and back fat

thickness. This is the first study to report significant associations between these SNP and marbling score and back fat thickness.

A significant association was reported between CAST SNP rs109906719 and rib eye area. Effects of markers on CAST for rib eye area have been previously reported (Schenkel et al., 2006). A significant association was reported between CAST SNP rs109020860 and back fat thickness. This is the first study to report significant associations between this SNP and back fat thickness.

The SNP found to be associated with growth and carcass quality and composition traits in this study are of interest for genetic selection of these traits in beef cattle populations. Further research should be conducted with larger, more varied populations and an increased number of SNP, candidate genes, and genomic regions in order to validate the potential usefulness of these SNP in selection strategies.

CHAPTER V SUMMARY

The studies presented in this thesis validate the variation expected between modern Charolais sired animals and multigenerational Angus sired animals. The Charolais sired animals had greater birth weights, larger rib eye areas, lesser back fat thickness measurements, and lower marbling scores as compared to the multigenerational Angus sired animals. The results observed are in agreement with previous studies and further validate the differences between the two breed types.

This is the first study to utilize multigenerational Angus for SNP associated with growth and carcass quality and composition traits. Utilization of candidate genes associated with growth and carcass quality and composition traits allowed for the evaluation of markers associated with multiple traits. The results observed indicate additional information about associations between SNP located on the CAPN3 gene not reported in previous studies. The results observed in the current study when evaluating the CAST gene are in agreement with a previous study that evaluated effects of markers on CAST with rib eye area.

Further research should be conducted to validate these SNP in populations consisting of other breeds. Furthermore, studies with larger, more varied populations, and an increased number of SNP, candidate genes, and genomic regions should be conducted to evaluate the usefulness of these markers located on the CAST and CAPN3 genes before they can be validated for use in marker assisted selection.

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APPENDIX A: DNA EXTRACTION – SATURATED SALT PROCEDURE

Based on extraction procedures described in Miller et al., 1988. Nucleic Acids Research 16:1215.

Day 1: in 15mL centrifuge tube

Add: 10-12 mL Lysis Buffer (Appendix B) to 250 μ L white blood cell buffy coat

Spin: 15 minutes at 4000 rpm at 4 °C

Aspirate supernatant from pellet

Add: 3 mL Digestion Buffer (Appendix B)

Resuspend pellet by shaking vigorously by hand

Add: 200 μ L 10% SDS Page and 60 μ L RNase A (10 mg/ml)

Invert to mix

Incubate 1 hour at 37 °C on shake setting

Add: 25 μ L Proteinase K (20 mg/ml)

Invert to mix

Incubate overnight at 37 °C on rotation setting

Day 2:

Add: 1 mL Saturated NaCl

Shake vigorously by hand for 15 seconds

Spin: 45 minutes at 4000 rpm at 4 °C

Transfer: Supernatant to new 15 mL tube, note volume

Add: 2 volumes of 100% Ethanol (stored in freezer)

Invert gently to mix

Remove DNA with soft pipet

Put DNA into 1.5 mL tube

Spin: 10 minutes at 10 setting in refrigerated centrifuge

Aspirate off ethanol

Add: 1 mL of 80% Ethanol (stored in freezer)

Shake on vortex ~20 seconds

Spin: 5 minutes at 10 setting in refrigerated centrifuge

Aspirate off ethanol

Add: 500 μ L of 80% Ethanol (stored in freezer)

Shake on vortex ~20 seconds

Spin: 5 minutes at 10 setting in refrigerated centrifuge

Aspirate off ethanol

Dry pellet overnight

Day 3:

Add: 350 μ L Load Buffer (Appendix B) to resuspend pellet

APPENDIX B: BUFFER SOLUTIONS

LYSIS BUFFER (1L):

7.49g NH_4Cl
2.059g trisHCl

pH to 7.4

DIGESTION BUFFER (1L):

1.21g trisHCL
23.376g NaCl
0.744g EDTA

pH to 8.0

REHYDRATION BUFFER (1L):

1.21g trisHCl
0.37g EDTA

VITA

Jennifer Lynn Bailey was born on March 13 to David and Linda Bailey in Slidell, Louisiana. In 2006, she graduated from Pope John Paul II High School in Slidell, Louisiana. Following her high school graduation, she attended Louisiana State University to pursue a Bachelor of Science in Animal, Dairy, and Poultry Sciences, which she earned in May of 2010. During her undergraduate studies in animal science, she became interested in research. After graduating, she began her graduate studies at Louisiana State University in beef cattle genomics under the direction of Dr. Matthew Garcia. She will receive the degree of Master of Science in Beef Cattle Genomics in May of 2013.