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## Evaluation of single nucleotide polymorphisms associated with fertility and production traits in Holstein and multi-generational Angus females

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EVALUATION OF SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED  
WITH FERTILITY AND PRODUCTION TRAITS IN  
HOLSTEIN AND MULTI-GENERATIONAL ANGUS FEMALES

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  
Rebecca Ann Hill  
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## **ABSTRACT**

The objective of this study was to test the association of single nucleotide polymorphisms (SNPs) with fertility in two populations consisting of Holstein cows and multi-generational Angus cows. The candidate gene approach was utilized and previously described SNPs were tested for possible associations with fertility. Single nucleotide polymorphisms on three genes were evaluated including leptin receptor LEPR, calpastatin CAST, and DGAT1. Fertility traits were evaluated in conjunction with production traits for Holstein females and growth traits for Angus females. One SNP was significantly associated with birth weight ( $P < 0.05$ ) in Angus females while a trend ( $P < 0.10$ ) was observed for two markers influencing birth weight performance and three markers influencing weaning weight performance. An association of two SNP for birth weight and back fat thickness in Angus females was identified. A trend ( $P < 0.10$ ) was observed for one marker within LEPR influencing average services to conception, two markers within CAST influencing average days open, two markers within CAST and one marker within DGAT1 influencing average protein production, and one marker within CAST and one marker within DGAT1 influencing average milk production. One SNP within LEPR was significantly associated with average milk production ( $P < 0.05$ ) in Holstein females. An association of one SNP within CAST and one SNP within DGAT1 for average protein production and average milk production in Holstein females was identified. An association of one SNP within CAST for average days open and average protein production in Holstein females was also identified. The association of these markers indicates that the evaluated quantitative trait loci (QTL) region may harbor causative mutations responsible for the variation observed in fertility and production

traits. Further evaluation of SNP in these regions is necessary in order to identify mutations accounting for the largest degree of variation for fertility and production traits.

## **CHAPTER I INTRODUCTION**

Selection for increased milk production in the dairy industry and meat production in the beef industry have been the primary emphasis of selection programs for many decades and traditional selection methods have led to a significant improvement in milk production and meat production. However, many studies have noted an antagonistic relationship between reproductive efficiency and production traits (McClure et al., 2010; Veerkamp et al., 2003). For instance, first-service conception rates in dairy cattle have declined from approximately 65% to 40% between 1951 and 1996 as milk production increased from 4500kg/year to 9000kg/year (Butler, 1998). The phenotype of Angus cattle has also evolved. Heavier, larger framed calves are now being produced when compared to those from the 1950s, 1960s, and 1970s. Enns and Nicoll (2008) reported an average 0.43 kg increase in body weight per year for Angus between the years 1973 to 1993.

Major consequences of declining fertility rates include increased culling rates, decreased longevity within the herd, and additional insemination costs. Higher culling rates lead to an increased cost in production and retainment of replacements. Generally these replacement animals are younger and less productive than proven females (Boichard, 1990). Additionally, heritability estimates in the literature for reproductive traits in beef and dairy cattle are low and extremely variable, ranging from 0 to 0.18 (Doyle et al., 2000; Buddenberg et al., 1989). Due to this low heritability, it is difficult to make improvements in reproductive traits using traditional methods of selection (Buddenberg et al., 1989).

Recently, genomic mapping utilizing candidate genes and genetic markers,

specifically single nucleotide polymorphisms (SNPs) have become an effective method to identify significant quantitative trait loci (QTL) in the genome. These markers will provide more accurate information, aiding in selection for economically important traits including milk yield, meat yield, and fertility. Detection of beneficial SNP genotypes for both production and reproduction within candidate genes would have the potential combined effect of improving production without decreasing an animal's fertility (Pimentel et al., 2010).

The research presented herein utilizes previously reported SNPs located within three known candidate genes leptin receptor LEPR, calpastatin CAST, and DGAT1 to test for possible associations with fertility. Fertility traits were evaluated in conjunction with production traits in a Holstein female population. Growth and production effects were evaluated in a modern and multi-generational Angus female population, potential effects on long-term fertility in Angus females who were kept as replacements were also evaluated.

## **CHAPTER II REVIEW OF LITERATURE**

### **Heifer Fertility**

Fertility is defined as an animal's ability to conceive, maintain pregnancy, and rebreed when mated at the opportune time relative to ovulation (Darwash et al., 1997). The definition of optimal fertility in heifers is the ability to conceive as a yearling, calve as a two year old and maintain a 12-month calving interval as an adult (Doyle et al., 2000). In order for conception and maintenance of pregnancy in heifers to occur, there must be synchrony between physiological process as well as management techniques (Dziuk et al., 1983). Management techniques include nutrition programs, genetic selection, reproductive management, and herd health. Proper reproductive management includes accurate heat detection or synchronization, which then allows for proper timing of artificial insemination thus increasing the likelihood of pregnancy (Wall et al., 2003).

In order for heifers to be efficient in a production scheme they must conceive early in the breeding season (Martin et al., 1992). Heifers who conceive as yearlings have greater longevity in the herd than their counterparts who fail to conceive as yearlings (Lesmeister et al., 1973). In addition, individuals who calve as two year olds have the potential to produce more calves during their lifetime versus heifers that calve for the first time as three year olds (Lesmeister et al., 1973).

Reproductive traits are of primary interest in livestock because they play a major role in production efficiency (Pryce et al., 2000; Doyle et al., 2000). Due to the long generation interval in cattle and low heritability of reproductive traits, direct improvement

through traditional methods of selection has resulted in minimal success (Buddenburg et al., 1989).

### **Fertility in Dairy**

Over the last several decades, genetic improvement of dairy cows has focused mainly on increasing milk production. During this time services to conception has increased while average conception rates have declined (Pryce et al., 2000). This trend is due to an antagonistic relationship between reproduction and milk production (Butler et al., 1989). Holtsmark and associates (2008) validated this trend by estimating genetic correlations in quantitative genetic studies by using 305-day lactation milk yield. This study concluded that dairy cows have a slight to severe negative energy balance during early lactation, which has a large influence on the postpartum start of luteal activity. Linkage disequilibrium, pleiotrophic gene effects, and other physiological associations may account for the negative genetic association between reproductive efficiency and production traits in dairy cattle (Veerkamp et al., 2003).

### **Multigenerational Studies**

Between 1965 and 2000 as the total cattle inventory steadily decreased, total commercial beef production steadily increased. According to Hughes (2002) this trend has been caused by an increase in beef production per cow. In 1980, an individual animal had the ability to produce 450lbs of carcass beef per year; in 2000 an individual animal had the ability to produce 620lbs of carcass beef per year. Over multiple generations, breeders have made significant changes in the stature, body composition, and growth of beef cattle through selection based on phenotype as well as expected progeny differences (EPDs) (Wilson et al., 1993).

## **Genetic Markers**

Genetic markers are defined as tools that involve specific alleles or loci within a genome that can act as regulators or indicators for specific functions or traits. These markers can then be utilized to identify specific regions of chromosomes where genes affecting quantitative traits are located (Davis et al., 1998) and typically involve variants in genomic sequence. These differences are detectable via enzyme digestion or other biochemical techniques and may or may not cause a phenotypic change (Vignal et al., 2002).

There are multiple genetic markers utilized in genotyping including microsatellite markers, single nucleotide polymorphisms (SNP's), amplified fragment length polymorphisms (AFLP's), and restriction fragment length polymorphisms (RFLP's). A microsatellite is defined as a sequence in tandem repetition, and is commonly used in QTL mapping (Sellner et al., 2007). A single nucleotide polymorphism (SNP) is a DNA sequence variation that occurs when a single nucleotide in the genome differs between individuals. These sequence variations have the ability to alter the amino acid produced at a specific location within a chromosome. In order for a sequence alternative to be considered a SNP, the least frequent allele must have a frequency of at least 1% in the population. Single nucleotide polymorphisms can occur in the coding and non-coding regions of the genome (Vignal et al., 2002).

A restriction fragment length polymorphism utilizes DNA restriction enzymes to digest DNA and identify allelic variation linked to a trait of interest. Restriction fragments may then be displayed in agarose gels via electrophoresis, yielding DNA fragments of differing molecular size (Botstein et al., 1980). Restriction fragment length

polymorphisms are easily assayed in individuals, making them ideal for studies utilizing large populations (Vignal et al., 2002).

Amplified fragment length polymorphism is a DNA fingerprinting technique that utilizes polymerase chain reaction amplification in order to detect genomic restriction fragments on DNA fragments. Rather than displaying fragment length differences, amplified fragment length polymorphism technique will display either a presence or absence of restriction sites (Vos et al., 1995).

The discovery of genetic markers for reproductive traits have given producers a method to potentially identify superior animals, improve selection response, and further enhance economic gains (Allan et al., 2008). Genetic regulation of quantitative traits is extremely complex. Therefore, a large number of genetic markers are required in order to identify genes that underlie genetic variation. According to Ramos and associates (2009) the limiting factor when analyzing economically important traits in livestock is the lack of an adequate number of genetic markers to develop high-throughput and high-density assays for association studies.

### **SNP Association Studies in Fertility**

The goal of SNP (single nucleotide polymorphism) association studies is to evaluate SNPs as potential sources of variation that predispose individuals to perform superiorly or detrimentally for particular traits. Previous research conducted by Pryce and associates (2010) identified SNP markers associated with milk production and female fertility. Single nucleotide polymorphisms were tested in an initial population of 780 Holstein sires and validated in 364 Jersey sires and 386 Holstein sires. The results of this study indicated that correlations between fertility and milk production traits were



unfavorable in Jersey and Holstein breeds.

Additional research conducted by Pimentel and associates (2010) utilized SNP association studies to analyze production and fertility traits in Holstein cattle.

Association analyses of production and fertility traits were conducted utilizing SNPs located near 170 candidate genes previously derived by Bauersachs and associates (2005) and Mitko and associates (2008). Sixteen SNPs significantly effected (5% false discovery rate) fertility and production traits, four of which contributed to a favorable relationship between fertility and productivity. The SNP that contributed favorably to fertility and productivity were located on the TNFSF10, PARP12, APBA1, and SCRN1 genes. The TNFSF10 gene, which is known for upregulating mRNA in the bovine endometrium at day 18 gestation, significantly affected fat percent, fat and protein yield, and interval to first successful insemination. The PARP12 and APBA1 genes significantly affected fat and protein yield as well as interval to first successful insemination. The SCRN1 gene significantly affected protein and fat percentage, fat and protein yield, and 56-day cow non-return rate.

### **QTL Approach**

Developments in technology have enabled the detection and analysis of microsatellites, which assist in the identification of regions of interest in the genome that potentially influence quantitative traits (Dekkers, 2004). These regions are termed quantitative trait loci (QTL). The quantitative trait loci approach investigates possible linkages between trait variation and genetic markers in a segregating population. This allows for the detection of genomic regions that may influence a trait of interest. Identification of these regions enables fine mapping of QTL regions that could lead to

the identification of candidate gene(s) well as the polymorphisms driving variation observed in the trait of interest (Mormede, 2005).

### **QTL Association Studies in Fertility**

Ashwell and associates (2004) utilized the QTL approach to identify QTL affecting female fertility as well as milk production traits. In this study, genotypic data from 367 markers in Holstein grandsire families was collected. Data analysis indicated that putative QTL associated with pregnancy rate were significant on six chromosomes: BTA 6 (*Bos taurus* autosome 6), BTA 14, BTA 16, BTA 18, BTA 27, and BTA 28. Evidence from this study also indicated that in addition to pregnancy rates BTA 6 affected protein and fat percentages and BTA 14 affected fat percentage. Therefore, it is possible for QTL regions significantly associated with fertility to overlap with QTL regions significantly associated with production traits.

Pryce and associates (2010) conducted a genome wide association study for fertility and production traits in the Holstein and Jersey dairy breeds. This study identified novel quantitative trait loci (QTL) regions including a putative QTL for fertility located on chromosome 18. This region was detected using haplotypes greater than 3 SNPs in length. Results from this research indicate that QTL regions associated with fertility overlap with QTL regions associated with production traits. These results may assist in identifying useful markers for QTL detection and marker-assisted selection for improvement of economically important traits.

### **Candidate Gene Approach**

The candidate gene approach utilizes genes of known physiological function in order to identify genetic variations associated with a phenotype of interest (Pimentel et

al., 2010). This approach has been very useful in detecting loci even with small effects provided that the candidate gene represents a true causative mutation (Andersson, 2001). Rothschild and associates (1996) utilized this method to determine the effects of the swine estrogen receptor gene in relation to increased litter size. In this study, two breeds of swine known for differences in prolificacy were compared and genotyped using inherited restriction enzyme sites. Sows homozygous for the BB genotype produced on average, 1.5 more piglets per litter when compared to sows of AA and AB genotypes. Due to its relationship with high estrogen levels and multiple ovulations as well as potential increased litter size the estrogen receptor gene was determined to be a useful candidate gene.

### **Leptin Receptor (LEPR) Gene**

The leptin receptor gene is located on BTA 3 (Pfister-Genskow et al., 1997). The leptin receptor gene is a member of the cytokine I family of receptors and signal transducers. Previous studies have identified the leptin receptor gene to be expressed in a variety of tissues in ruminants including the mammary glands and liver (Bartha et al., 2005). In ruminants, expression of the leptin receptor gene is affected by an animal's level of nutrition (Chilliard et al., 2005). Additionally, blood leptin concentrations interfere with luteinizing hormone secretion and stimulate growth hormone release (Kadokawa et al., 2006; Nonaka et al., 2006). In both beef and dairy breeds the leptin receptor gene polymorphisms have been described as affecting milk yield, live weight, feed intake, and fertility (Schenkel et al., 2006; Clempson et al., 2011). These associations may provide insight into the underlying mechanisms of the leptin receptor gene and results could be utilized in future breeding programs (Almeida et al., 2008).

### **Calpastatin (CAST) Gene**

Calpastatin is an endogenous and specific inhibitor of m-calpain and  $\mu$ -calpain and is involved in the degradation of myofibrillar proteins in post-mortem proteolysis, which directly effects post-mortem meat tenderness (Casas et al., 2006). The calpain-calpastatin system has been described to increase the rate of skeletal muscle growth as a result of decreased muscle protein degradation. The process of decreased muscle protein degradation has previously been shown to be directly associated with an increase in CAST gene expression (Kubiak et al., 2008).

Calpastatin is a protein encoded by the calpastatin gene (CAST) located on BTA7 (Bishop et al., 1993; Raynaud et al., 2005). In 2006, Garcia and associates identified a mutation in the CAST gene associated with daughter pregnancy rate (DPR) in Holstein cattle. The predicted transmitting ability for DPR was +0.13 for the wild type genotype, -0.44 for the heterozygous genotype, and -0.69 for animals that were homozygous for the mutant genotype. Identification of genetic mutations such as this has the potential for direct selection of animals possessing gene variants superior for both milking ability and fertility.

### **Diacylglycerol Acyltransferase (DGAT1)**

The AcetylCoA:Diacylglycerol acyltransferase DGAT1 gene has been mapped to the centromeric end of BTA14. DGAT1 is a microsomal enzyme that plays a central role in the biosynthesis of cellular glycerolipids. DGAT1 further catalyzes the final step in triacylglycerol synthesis by using diacylglycerol (DAG) and fatty acyl CoAs as its substrates (Cases et al., 1998). Grisart and associates (2002) identified a lysine/alanine polymorphism K232A located in exon 8 of the DGAT1 gene. The lysine allele was

associated with increased fat yield and protein percent. Variations in the fat:protein ratio in milk during early lactation has previously been described as having negative effects on fertility (DeVries et al., 2000). In addition to its effects on fertility, DGAT1 is considered a candidate gene for intramuscular fat deposition. As with fat yield and protein percent, the lysine allele is consistently the more efficient version of the enzyme in regards to triglyceride synthesis. (Cases et al., 1998; Winter et al., 2002).

### **SNP Chip & IPLEX Technology**

The most efficient method for genotyping large numbers of SNPs is through the design of a high-throughput assay that includes a large number of SNPs. These high-density panels are referred to as ‘chips’ and are a valuable resource for genetic studies in livestock. Some of these studies include genomic selection for economically important traits (Ramos et al., 2009), QTL identification, comparative genetic studies, and parentage testing (Illumina Inc., San Diego, California). High-density SNP genotyping has become readily available as a biomedical diagnostic for predicting predisposition to heritable genetic diseases. Similar applications of this technology have become readily available in cattle leading to improved herd health, increased animal productivity, and increased selection accuracy (Matukumalli et al., 2009).

Currently, there are multiple platforms available for use in whole genome association studies. These technologies utilize probe labeled primers in order to distinguish the two alleles of a SNP (Myakishev et al., 2001). In order to effectively utilize this technology it is vital to determine the chromosomal position of the SNP of interest prior to its utilization (Schmitt et al., 2010). The chip itself contains over 700,000 SNP markers uniformly distributed across the genomes of various cattle breeds

(Illumina Inc., San Diego, California).

The Sequenom MassARRAY (Sequenom, San Diego, California) system allows for SNP analysis in low and high sample throughput applications. The MassARRAY system is used for linkage studies, genetic testing, and fine mapping of SNP. The MassARRAY software designs iPLEX and polymerase chain reaction (PCR) single base extension primers to be used for multiplexed assays.

### **Marker Assisted Selection**

Marker-assisted selection (MAS) in livestock selection programs allows for increased accuracy of selection of specific DNA variations that are associated with measurable differences in economically important traits. The rate of genetic improvement achieved by MAS may be substantially greater than improvement achieved by selection based on EPD values for traits that are lowly heritable or determined post-mortem (Wilson et al., 1993). Therefore, marker assisted selection has the potential to greatly increase the efficiency of animal breeding (Davis et al., 1998).

Previous research conducted by Davis and DeNise (1998) observed that there are three phases in the development of MAS programs. First is the detection phase, followed by evaluation phase, and finally the implementation phase. The first phase is the detection phase in which DNA polymorphisms are used as direct or linked markers in order to detect specific allele frequencies within QTL segregating populations. During this phase markers associated with QTL are identified and the size of the QTL allele effects and the location of the QTL within the genome can be estimated. In the evaluation phase linked markers are tested in target populations to determine whether QTL segregated within the population. Finally, in the implementation phase predictive

linked markers in a population are used within families and direct markers are used across families in order to produce a genotypic database. These data are combined with pedigree and phenotypic information in genetic evaluation to predict individual genetic merit.

### **Whole Genome Selection**

Whole genome selection (WGS) is a form of marker-assisted selection that utilizes genetic markers distributed throughout the entire genome so that all QTL are in linkage disequilibrium with at least one marker (Andersson, 2001; Goddard et al., 2007). The objective in using whole genome selection is to utilize genomic data to supplement extensive sets of performance data in order to predict genetic merit values so that producers can make informed selection decisions (Matukumalli et al., 2009). An advantage to utilizing this method of selection is that it allows for the prediction of additive genetic value for epistatic and pleiotrophic effects of alleles known as haplotypes for each chromosomal region that is influencing the trait of interest. Summing across all loci affecting a trait the genetic merit of an animal can be predicted based on the multi-locus genotype (Daetwyler et al., 2007).

Animals with phenotypes or predicted additive genetic merits can be genotyped at a high density with over 775,000 SNP distributed evenly throughout the genome. Either individual SNP or chromosomal regions containing haplotypes are analyzed as independent random effects under a mixed linear model to simultaneously determine genomic regions contributing to phenotype as well as predict the additive values of each haplotype within each region. The phenotype or genetic merit of an animal can be predicted based solely upon its genotype information from predicted haplotype values

(Sellner et al., 2007). In order to avoid estimating a large number of variance components for regions and to make the approach statistically tractable, Meuwissen and associates (2001) assumed equal variances associated with each chromosomal segment as well as independence between regions. McKay and associates (2007) determined that these assumptions are violated by the existence of long-range linkage disequilibrium and because those regions closest to QTL will contribute much more variance to a trait than the rest.

### **Mixed Model**

A mixed model is a nonlinear statistical model that accounts for both fixed and random effects in the statistical model (SAS Institute Inc., Cary, North Carolina). Mixed models have a multilevel, hierarchical structure and are often utilized in animal breeding applications because of their ability to estimate genetic and phenotypic variation or to predict the genetic merit of selection candidates. Observations made between clusters are independent, but observations within a cluster are dependent since they belong to the same subpopulation. Therefore, two sources of variation exist: between clusters and within clusters (Liu et al., 2008).



# **CHAPTER III**

## **EVALUATION OF CANDIDATE GENES AND SUBSEQUENT EFFECTS ON CARCASS TRAITS AND FERTILITY IN MULTI-GENERATIONAL ANGUS AND MODERN ANGUS FEMALES**

### **Introduction**

Dramatic improvement in the Angus breed for carcass quality and composition traits has been observed over many years (Northcutt et al., 1993). The mature weight for Angus cows ranging from five to 12 years in age increased from 519kg in 1963 (Northcutt et al., 1993) to 630kg in 2011 (McHugh et al., 2011).

While advances have been made in growth and production traits in Angus beef cattle, fertility rates have declined. Parnell and associates (1997) observed that yearling weights for Angus females comprised of lines selected for high and low yearling growth rates were 2.11 kg and -2.54 kg, respectively. During this time, it was noted that high line females achieved puberty at an earlier age and had significantly longer gestation periods than low line females (Archer et al., 1998).

Three known candidate genes leptin receptor LEPR, calpastatin CAST, and DGAT1 were selected based on their previously recorded associations with fertility and production traits in Angus cattle (McClure et al., 2010; Pintos et al., 2011). The objective of this study was to evaluate the growth and production effects of a modern Angus female and multi-generational Angus female population as well as potential effects on long term fertility in females kept as replacements.

### **Experimental Animals**

Twenty-five modern sired purebred Angus females and twenty-two multi-generation sired purebred Angus heifers produced from modern purebred Angus cows were utilized in this study. Multi-generational calves were produced via artificial

insemination on modern Angus females utilizing frozen-thawed semen from thirteen sires born from 1960 through 2006. All Angus females had an average body condition score of five (0 = emaciated, 9 = obese). The Select Synch protocol (Geary et al., 2000) was utilized prior to artificial insemination with aged frozen/thawed semen to synchronize modern Angus females. Females that did not respond to the Select Synch protocol were given an injection of prostaglandin ( $\text{PGF}_{2\alpha}$ ) (Pfizer Animal Health, New York, NY) and artificial insemination was repeated during the next observed estrus.

Specifically, females evaluated in the current study were comprised of twenty-two multigenerational Angus females born in 2010, eleven modern sired Angus females born in 2008, eight modern sired Angus females born in 2009, and six modern sired Angus females born in 2011. Modern Angus females were produced via artificial insemination or a single pasture bull clean-up system.

All Angus females were born and managed at Louisiana State University Agriculture Center Central Research Station located in Baton Rouge, Louisiana. All females were maintained on natural pastures and developed until puberty on adapted Ryegrass pastures until first breeding. Individual animal weights were collected at birth and weaning. Ultrasound carcass traits were recorded at the conclusion of the present study.

### **Blood Collection and DNA Extraction**

Blood samples were collected from all Angus females via jugular venipuncture. Blood was transferred into 20mL tubes and centrifuged at 4000rpm at 4°C for 20 minutes. White blood cell buffy coats were extracted and transferred to 250 $\mu$ L micro-centrifuge tubes. Genomic DNA was isolated and purified from buffy coats using a

previously described saturated salt procedure (Miller et al., 1988) (Appendix A). Working solutions were prepared for genotyping by diluting extracted DNA to a concentration of 25ng/ $\mu$ L. Subsequently, a total of 500ng of DNA was removed for a genotyping solution. Extracted stock DNA and working solutions were stored at -4°C.

### **SNP and Genotyping**

Previously reported single nucleotide polymorphisms (SNPs) on candidate genes LEPR, CAST, and DGAT1 were collected from the dbSNP website (<http://www.ncbi.nlm.nih.gov/projects/SNP/>). Due to its direct involvement with triglyceride synthesis, DGAT1 is considered a candidate gene for intramuscular fat deposition (Thaller et al., 2003). Previous studies have identified LEPR to be expressed in a variety of tissues including the liver and mammary glands (Bartha et al., 2005). In addition, previous research suggested that polymorphisms within LEPR might affect subcutaneous fat and fat yield in beef cattle (Schenkel et al., 2005). Therefore, LEPR has emerged as a candidate gene in the evaluation of growth traits and carcass composition (Guo et al., 2008). Increased CAST gene expression has previously been described to decrease muscle protein degradation (Kubiak et al., 2008). Additionally, a SNP located on the CAST gene associated with daughter pregnancy rate (DPR) in Holstein cattle has been reported (Garcia et al., 2006). Therefore, CAST is considered a candidate gene for fertility and meat tenderness.

Single nucleotide polymorphisms were selected by identifying SNP that were evenly distributed over the entire length of each candidate gene. The justification for this selection method was to account for possible linkage associations with potential causative mutations located on the candidate genes. Selected SNP, forward and

reverse primers and allele substitutions for LEPR, CAST, and DGAT1 are reported in Table 3.1, Table 3.2, and Table 3.3. IPLEX reactions for all genes and SNP were generated by Illumina Inc. (San Diego, California). Single nucleotide polymorphism genotyping was conducted by NeoGen (Lincoln, Nebraska) utilizing Sequenom genotyping technology (Illumina Inc., San Diego, California).

### **Ultrasound Measurements**

Carcass quality and composition measurements were measured via ultrasound technology by a certified technician. Carcass traits were measured with a 3.5MHz linear probe utilizing Designer Genes BioProbe 1049 software (Harrison, AR) setting 90N-25F2.1. Measurements included fat thickness at the 12<sup>th</sup> and 13<sup>th</sup> rib, ribeye area, and intramuscular fat percentage. These measurements were calculated on a per 100lb basis.

### **Statistical Analysis**

Initial mean separation analyses were conducted on modern sired Angus female population versus multi-generational sired Angus female population to analyze potential differences in performance. Means were separated for birth weight, weaning weight, intramuscular fat, ribeye area, and back fat thickness using a two tailed T-test. The Mixed Model procedure of SAS (SAS Institute Inc., Cary, North Carolina) was utilized for statistical analysis. The model included fixed effects for sire, dam generation group, sire within birth year, and individual candidate gene SNP. Sire within generation group was also fitted as a random nested variable to account for potential confounding affects observed in the data. Dependent variables of birth weight, weaning weight, back fat thickness, ribeye area, and intramuscular fat were fit into the mixed model procedure in

**Table 3.1: Single nucleotide polymorphism ID, allele substitution, and forward and reverse primer sequences utilized for amplification and visualization of genotypes for LEPR**

SNP ID	Allele Substitution	Forward Sequence	Reverse Sequence
rs135977111	A/G	CTTCTGTTCTCTTCCTTGCAAAACATGTAA	CAAGCTCCCTGGCAGTGGGATTTCCAGACA
rs133145962	A/G	TATCTTTGGCAGGAATGCAATCAAATGTGT	TTAATCAGTCATGTCTGACTCTTTGTGACC
rs43347905	A/G	TTTTCTCTGTGTCTTTTAAATGTCCTAACA	AATTTATTTATGTAATAACTGCATTTA ACT
rs133109480	A/G	GGTTTACAGTCCATAGAGTCGCAAAGAGTC	GACATAACTGAGCTGCTAAGCTCAAGCACG
rs43347912	G/T	CTGGACGGCCAGGGGGTTCCCTGAACTAAT	TTTAAAGTCACCCTAGGAGTAGAACAGATA
rs43347914	A/G	AAGCTCTTCCCTGCCTTCCCTTTGATTTTT	CTCAGAAGCCATTTTCATAGTTCTAACATTG
rs43347917	A/T	TTTAACCAATCCATTGATTTTTAATGTATG	AGTGTAACATTTTCAAATATCAAGTGAAAA
rs136901371	C/T	GAGACAAGAGAGAAGAGTTCAGAATAAAAT	GGGCTTGATTAATGGAGCAGAATACTCAAA
rs43348634	A/G	CTAAGCTGCTAAGTCACTTCAGTCATGTCC	ACTCTGTGCGAACCCATAGATGGCCTCCCA
rs134577752	A/G	CTGAGCACACTTGTTTACTTTACAAATAAC	CATGTTTCTTCTCTCAAAATTTTAGTTGGT
rs135915491	C/G	AGCAGCAAAGTGGTTTGAAAAATTGAAGTA	ATAGTGATCCTCAAGATGTTTTGTGTGCAT
rs43348652	A/G	TCTCTGCCCAGTATTGTCTACCCCTGCTCT	TGAGGCAGGAACCTTTGTCTCACTCACCATT
rs134375381	G/T	CAAAGACAAGAGCCTTTTGCTTGGAGTAAT	AAGGTAGGAGAACATTCAGAGATGTGGTTA
rs135560721	C/T	TTTTGAGGAGATTCAGTCATACTTCAATAT	GTACATTCAAGCTTTCATTCAAGATCAGCA
rs137541136	A/G	GCTATTTCAAATCCTAAAAGATGATGCTGT	AAAGTGTGGCACTCAATATGCCGGCAAATT
rs43348655	C/T	ACAGTCCATGGGGTCACAAAGAGTTGGACA	GACTGAGCAAAATCACTTGGTGCTGCATAA
rs43348659	A/C	AAGAATAATATTTTAGAGAAATATTGATTC	CCTTGTCTCGCCACACGACACTGGCACTG
rs137111668	C/G	CTCTCCTTATTAGAAAATTGTCATTTACTT	AATTGCATACCCACTTACTGTCAAGCAAAA
rs137842817	G/T	AAAGTTTAATTGGATGTTCTGATGGTTTTT	AAATCTGAGTAGTCATAACTCAAAGCTTAG
rs135263435	A/G	TACTAGAAGACACTGTGAAAATTCAACTTT	GGAATGACAGCTCCTCATTTTACTAGCTTT

**Table 3.2: Single nucleotide polymorphism 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
rs43529864	G/T	GTGGGAGCCAGCTCGGACGTACACGTGCTA	TCGGCGTGAGTTCAGGCTCACAAGTTGAAT
rs133108534	C/T	TTGTCCTATTTTTGATTGCAATGATTCTTT	TTCAGCCTCCTCAAGTCTGCCTTTGAATCC
rs134804900	A/G	TCTGAGTGAAATGTCTCCTACTTTAGGACC	GCATCCTGCACTTCCTGTCTTTGCTCCCGT
rs109727429	C/T	AGCTGGCTGACAGAGAGGAGAGCCAGGCTT	GCCCTGCTCCCGTGACATAAATCACTGCAG
rs133978255	G/T	CACAGAGTCGGACACGACTGAAGCGACTTA	CAGCAGCAGCATACTCTTAAGTAGTATCCA
rs135802918	G/T	AATTGGTCATTATATCACCACTGCCTAGAG	AGGACCAGGCTTCTAGCCAGGGTTCAGTAA
rs134187714	C/T	AATCCCATGGACAGAGGAGCCGCAAAGAGT	GGACAGGAATGAGCCACTTCACTTTCACTT
rs135598419	A/C	AGAGCGGTGCTTTGTATCTGTCTTTCAAGA	TGCAAAGTGTTTTCTGTTGAGATTTGACAGT
rs133440731	A/G	GGGTCACAAAGAGTCAGACATGTCTCAGCA	TCAGACAAACAGCAAGGGTGTTAATGCTTG
rs135336850	C/T	ATTCAGTGTTGGCTGAAATTCTACCGGTCT	GAGTCCAGAGTCCGCTCTCGCTCTCTTAGC
rs137673193	C/T	CAATTGCACCTGTGGAAGGACAGTCATTAA	ATATAGATAGTGAAAGTGAAACTGTTAGTT
rs110972443	A/C	CATCTGTTGATAGACTTATAGGTTGCTTCC	TGTGTTGGCTATTGTAAACAGTGCTTCAAT
rs134668965	G/T	TTATTGTTTTTCAGACTGTTGCTAGGATTAT	ATCAACCAGACACCAACAGCCATTTCTCTC
rs133997237	C/T	AATGAATAAAAGAGCACAGGGCAATCCGTT	ATGAGATGCATTTTATTTGGAAGAGGTGGA
rs133149410	A/G	TAATGTCTCTGCTTTTTAATACCAGGGAAT	TGTTAAATTTCTCTAGAAAGCTAGCAAAC
rs110647227	A/G	TCCTTAGGCATTCAAGAAAATCATGCTCAC	GCGGGTAGGGTAGCAGACYGTATTGTTGGT
rs109491082	G/T	TACAGAGATCGGGCTTCTGAGTCTCATGTT	TCCACCCGGTTTCCATTGCCAAGGACCAAG
rs111010631	C/T	ACACACTGAAGGAGCTTAATATATTGTTGC	TTATTAGAATTGAAGTGCAATAATGCATAT
rs133820366	A/C	AAGGCCTGCTGTCTCTCTTTCTTCCCCAAC	CCACCACCACCGGTGCTGTTGAGAACGAAG
rs136073124	C/T	GCCCTGTGTTTGATTCTACTTTACAGTAAC	GAAGAGCTGGTTTGGATGAGGGAGACTCTG

**Table 3.3: Single nucleotide polymorphism ID, allele substitution, and forward and reverse primer sequences utilized for amplification and visualization of genotypes for DGAT1**

SNP ID	Allele Substitution	Forward Sequence	Reverse Sequence
rs134049142	A/G	GGCACCCCTGTATGATGAGGGGCATGTGCCA	AGGGTGCCTGTGGCGAGCTCCCCACCTTGC
rs135576599	A/G	CCCCAGGGGATTCATGCAGGGAGGCCGTAG	AGCAGGCAGGGCCAGATGCCCAGCAAGACC
rs109711965	G/T	TGCCTGCCCTTTGGTGTGGCAGCCCCTTCA	GCCTCACCTCAGCCTTGGCGCCGGCAGCCT
rs134455341	A/G	GGAAAGGGAGTGGAGATGACCTTGAACACC	TGTCCTTTGCTTTTCTCGGGTCTCTGACCC
rs134374261	A/C	GCACAGCCGGGCGCGCAGCAGCTGTCAGCCC	CCTGCCGCCCCTGCAAGTCCTGTCTCCCCA
rs137617619	A/G	TGCCCGACTCCTGTGACCCCATGGATTGTA	CCCACCAAGCTCCTCTATCCATGGGATTTT
rs135048973	C/T	ATTGCCACCTAGGAAGCCCCCCCCCCCCACC	CCTTTGAATATTCTTGTCTCTTTTCCTTGT
rs136875432	A/G	TGCCCCCCTCCTCTTCGGGAGACCATGCAC	TTCTACGCAGCCTGGCACATCTGGCAGACA
rs132679620	A/G	TCCTGGGGCCTCGGGGGCAGAGTGTGTGTT	TGCAAAGACAAGGCCATCTGCCAGCAACCC
rs132778108	C/G	AGGAGCTGCAGCTTCGGCACCCCCCAACCC	CCCCCGCCACTCACCTCGGGTAGGTTCT
rs109701809	A/G	CTGTCTGCCCCGCGGGGGTATGTGTATCCTG	TGTCGTGTCCCGGGTTTGCTTGGCCCCCTCC
rs134718967	C/G	GTGCTCCCTCAACCTCAGGGGGCACTCGGGT	ACACCGGGCACAGTCAGGTTAGCAACCCCC
rs109663724	A/T	GTGCTGAACCACGCGCGTGGCGTGTACCAT	TCTCCATCCAGGGCCGCGACCGTGTGTCAGG
rs135423283	G/T	GCTGCTGTGGGAGCAGAGAAGTCACTTCGG	TTCCTGTCAGGGTTTTTCCTCAGGGCCATG
rs132669273	C/T	CACGAATGTAAGTAGCCCACCACAGTCCAC	ATCTGGCTCCTCCCAAGACCTCCAGCATCT
rs109169510	A/T	GGCTAAGGGGATGTTCTGCCCAAAAAGGA	GCAGGCAGGGTCTGGTGGGACTTCCTAGTA
rs137584522	C/G	AGATGAACCGCTCGGCCGAGGGGGATCCCT	CCCCACCCCCACTGCGGTCCCGCCGGCTG
rs132699547	C/G	GGCCGCCCACCTATCGGGGCAGAGGCAGTA	CAGTGCCCCCATCCCTGGAGCAGGGTCAGG
rs134110051	A/G	ACGGCCGCTGGGCAGCAGGTTTCTTCTGCC	CGGTGGCACAGGCACCTGGGGTTGTGGTTG
rs135143198	C/T	GGGGCTCAGCTCACTGTCCGCTTGCTTCCT	CCCCAGCTGTTCTCACCAGCTCCAGGTG

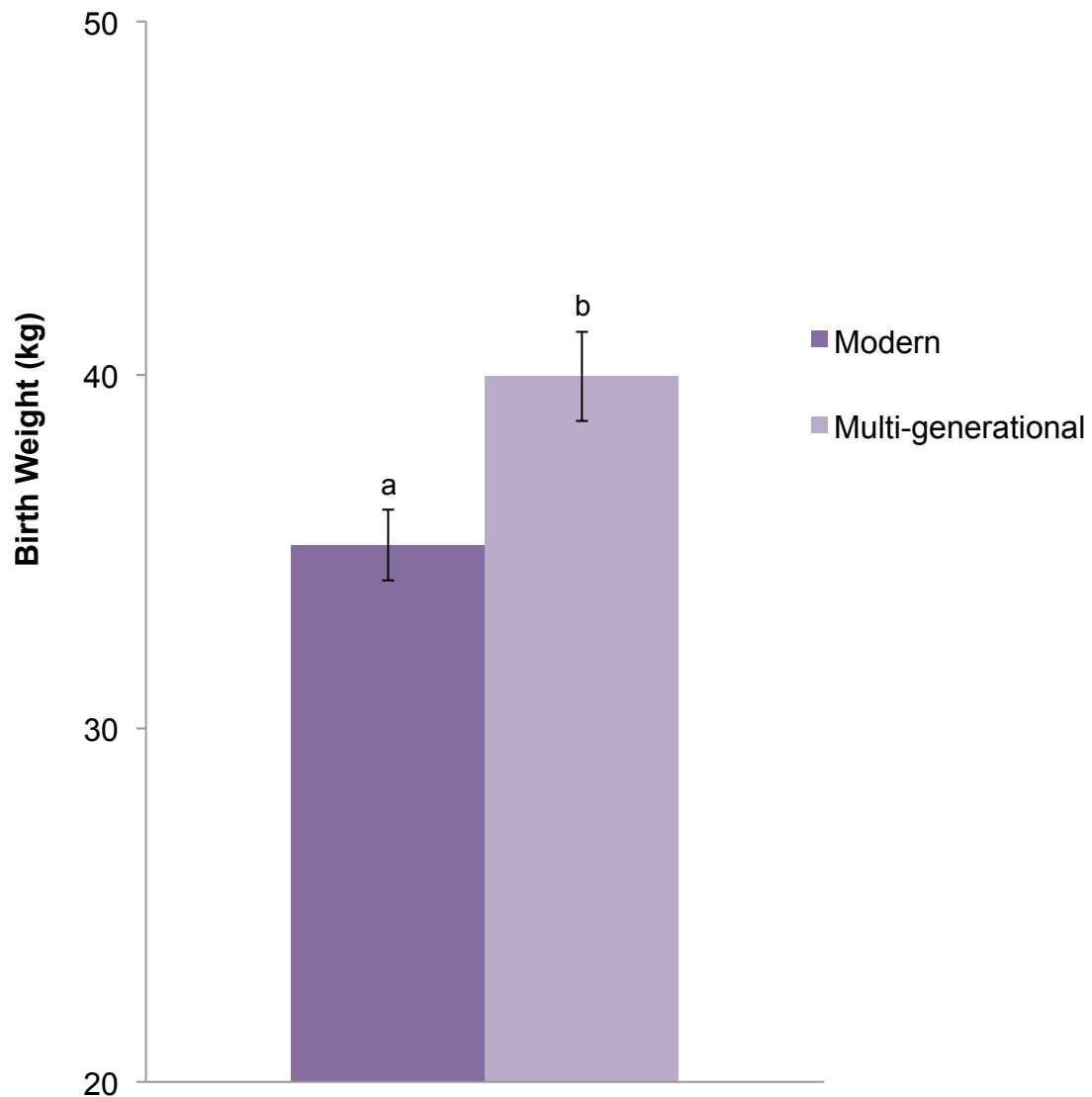
order to test for associations between SNP and the previously described traits. All statistical analyses were conducted using similar methodologies reported in previous studies (White et al., 2005). Single nucleotide polymorphisms with more than one genotype represented were included in the analysis. Any SNP with only one genotype were excluded from the analysis due to a lack of marker effects. Due to a limited sample population statistical significance was evaluated at ( $P < 0.05$ ) and statistical trend was evaluated at ( $P < 0.10$ ).

## Results

Mean birth weights were significantly higher ( $P < 0.05$ ) for multi-generation sired Angus females ( $39.96\text{kg} \pm 1.26$ ) when compared with modern sired Angus females ( $35.19\text{kg} \pm 1.00$ ) (Figure 3.1). Evaluation of weaning weights revealed that modern sired Angus females were significantly higher ( $P < 0.05$ ) ( $216.93\text{kg} \pm 6.12$ ) than multi-generation sired Angus females ( $198.74\text{kg} \pm 5.58$ ) (Figure 3.2). Mean intramuscular fat was significantly higher ( $P < 0.05$ ) for modern sired Angus females ( $5.40\% \pm 0.35$ ) when compared with multi-generation sired Angus females ( $4.33\% \pm 0.27$ ) (Figure 3.3). Mean ribeye area was not significant between modern sired Angus females and multi-generation sired Angus females (Figure 3.4). Mean back fat thickness was significantly higher ( $P < 0.05$ ) for modern sired Angus females ( $0.21\text{cm} \pm 0.025$ ) than for multi-generation sired Angus females ( $0.11\text{cm} \pm 0.018$ ) (Figure 3.5).

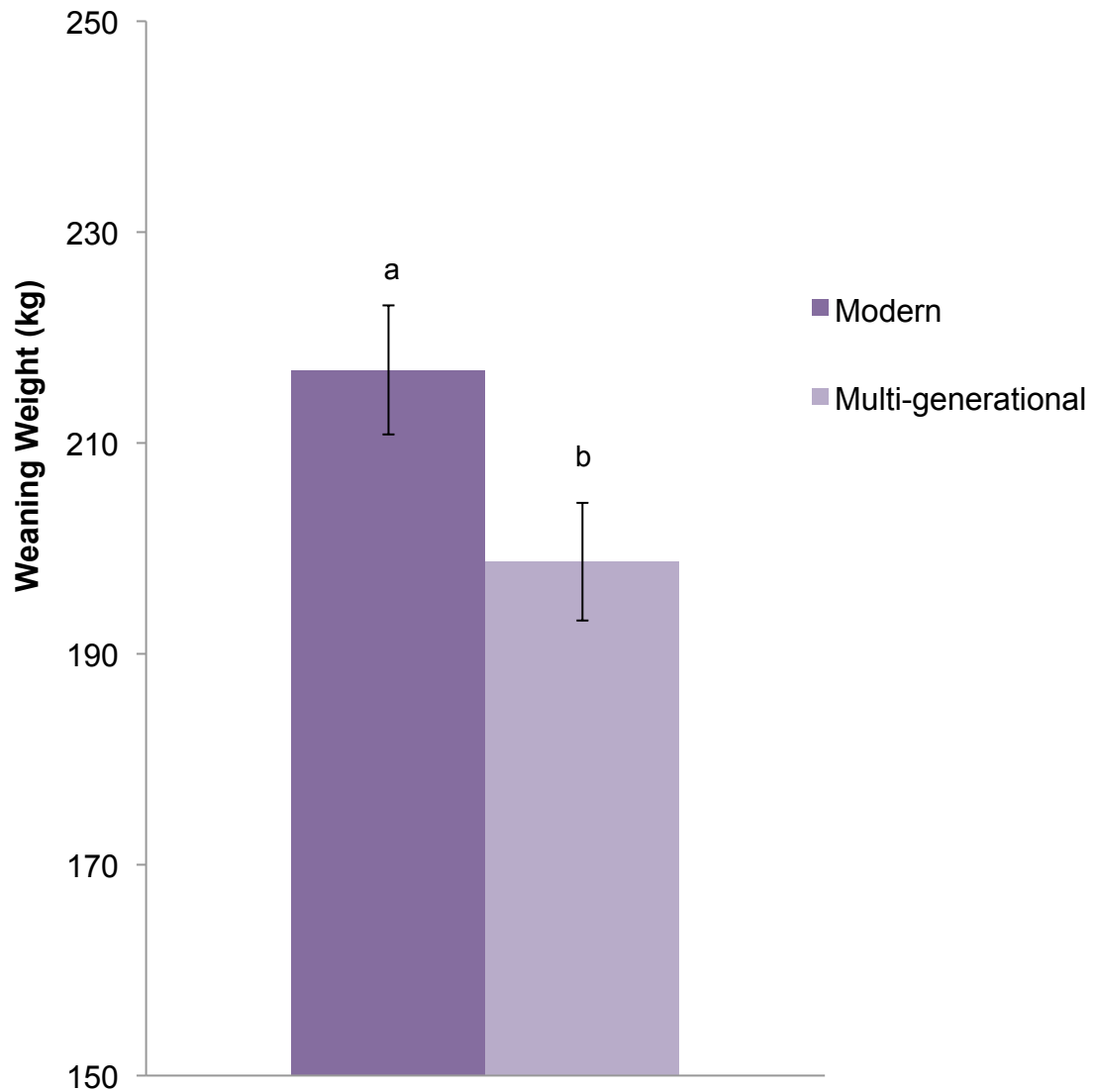
Three unique SNP located within LEPR were associated with birth weight (rs135263435, rs43348659, and rs134375381) (Table 3.4). Marker rs135263435 significantly ( $P = 0.03$ ) influenced birth weight performance. Animals inheriting the homozygous genotype GG for marker rs135263435 had birth weights that were higher





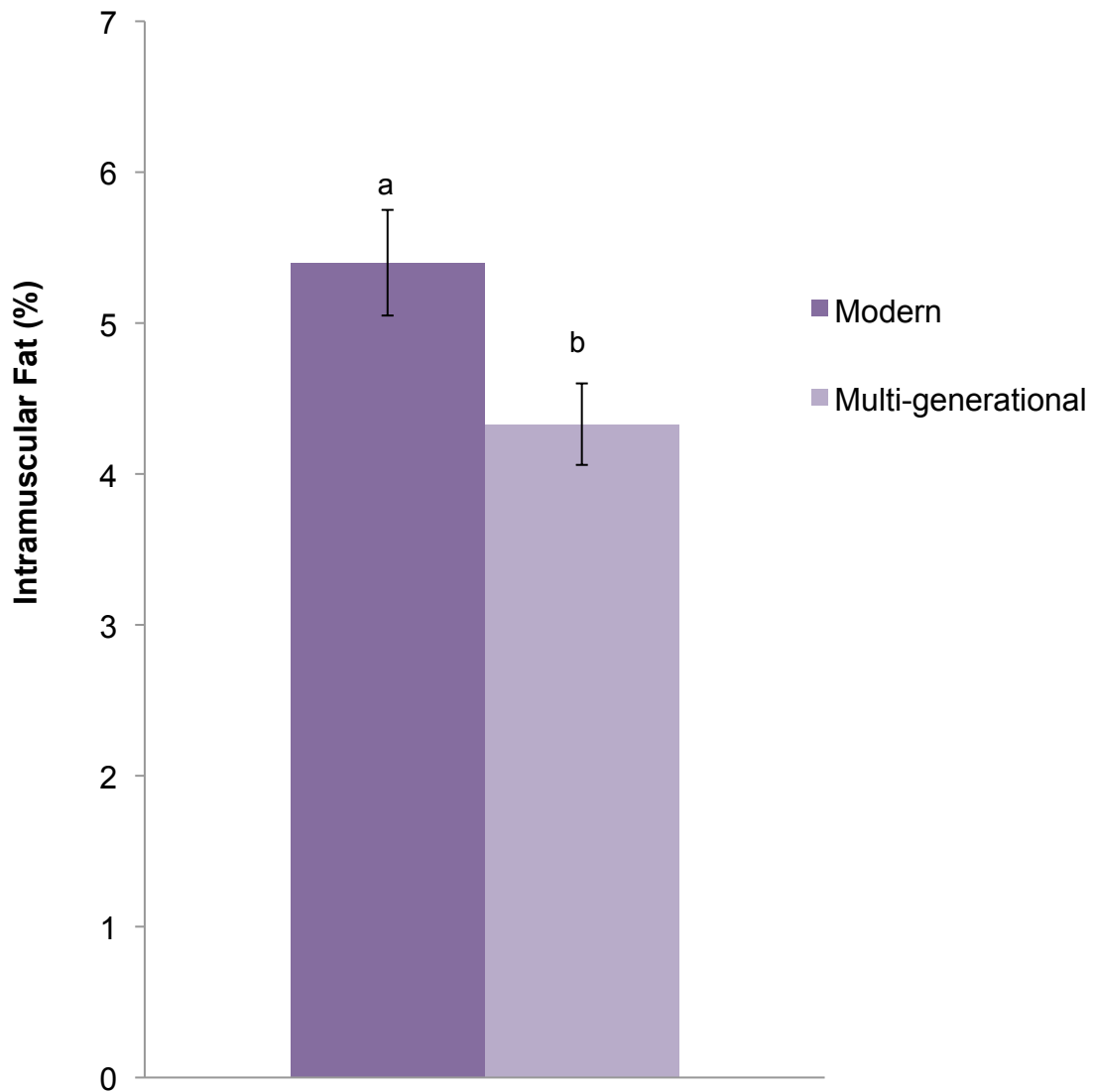
**Figure 3.1: Means separation analysis comparing modern sired Angus female and multi-generational sired Angus females for the trait of birth weight**

<sup>a,b</sup> Superscripts indicate significance at  $P < 0.05$



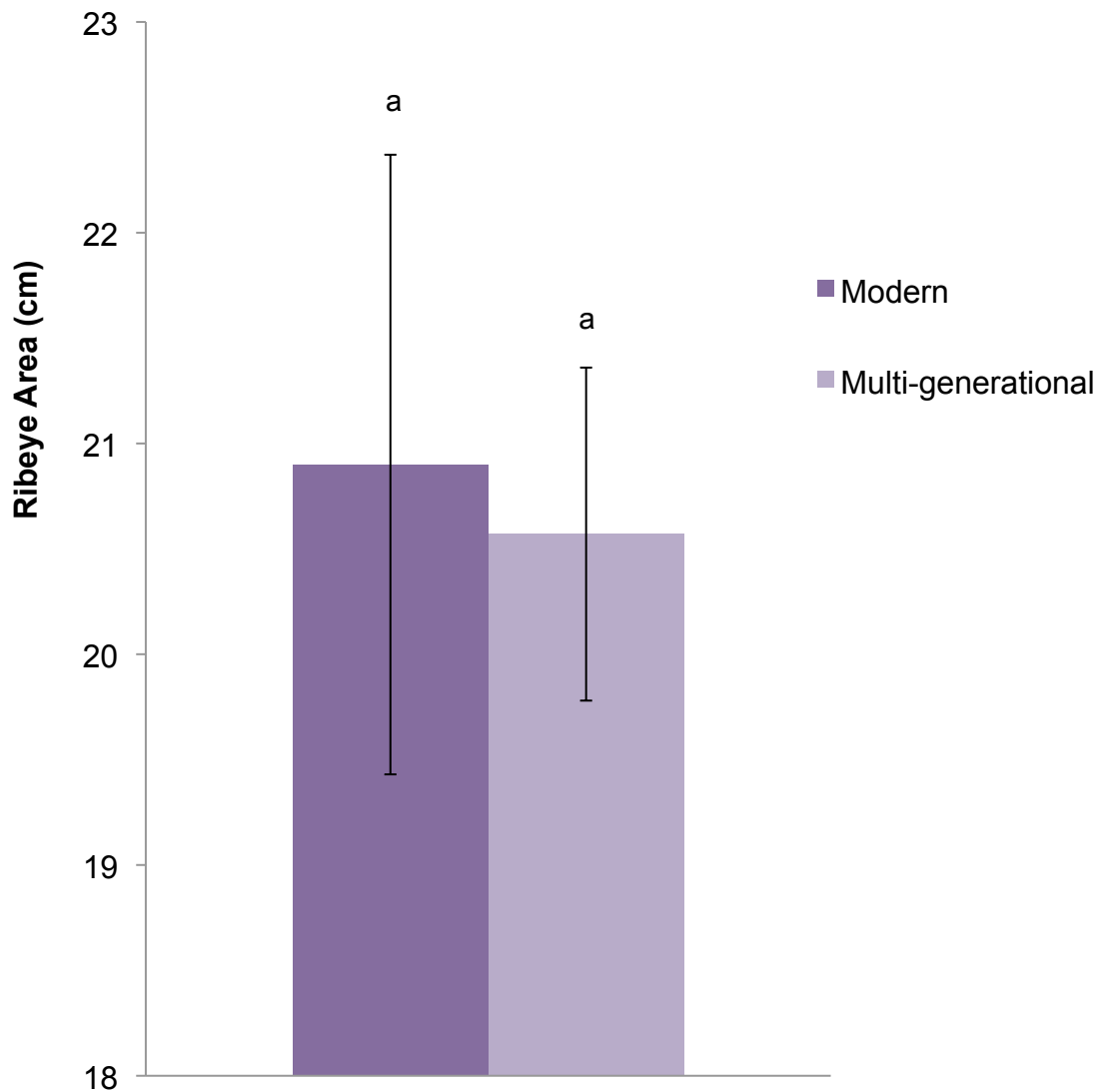
**Figure 3.2: Means separation analysis comparing modern sired Angus female and multi-generational sired Angus females for the trait of weaning weight**

<sup>a,b</sup> Superscripts indicate significance at  $P < 0.05$



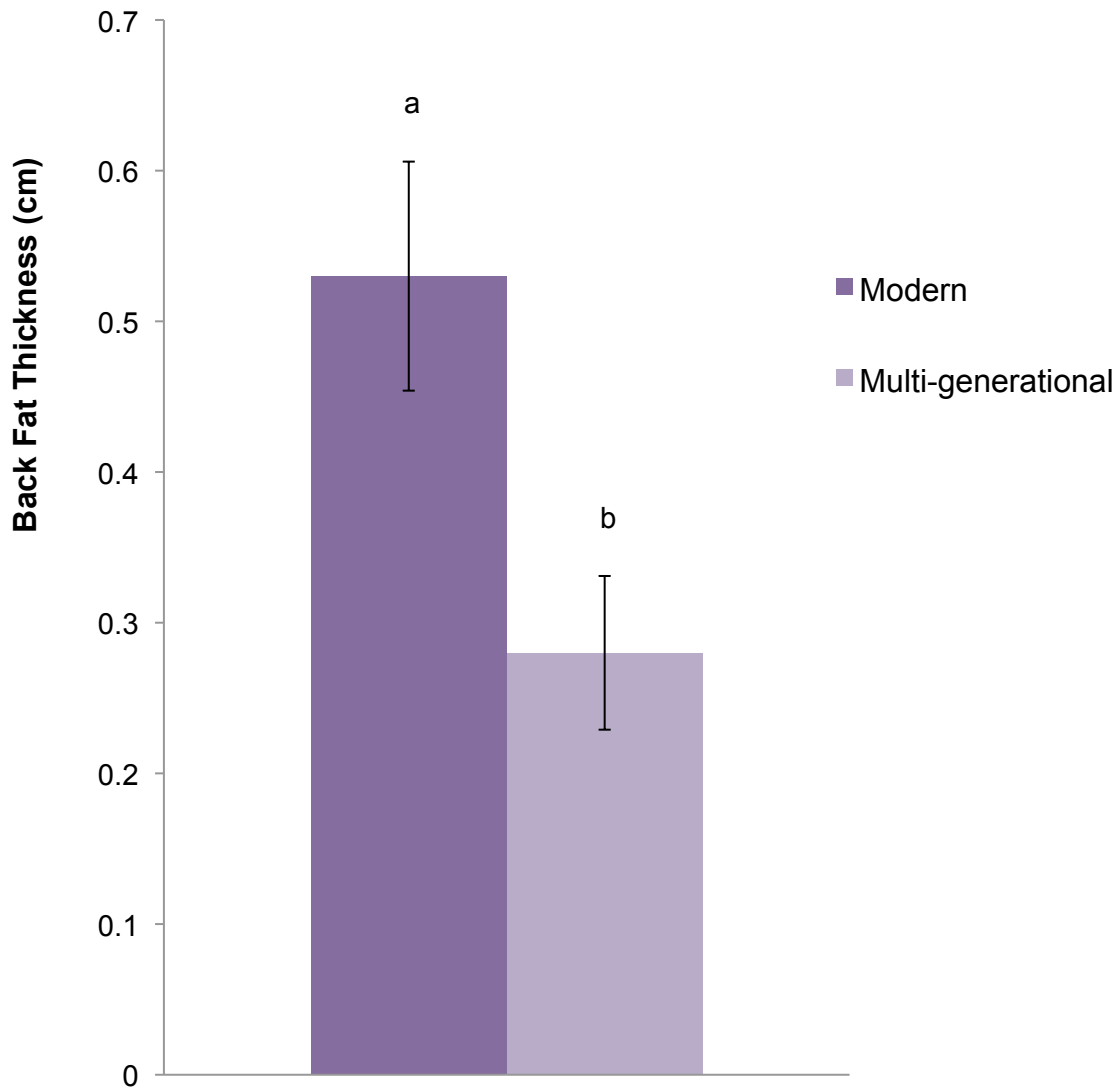
**Figure 3.3: Means separation analysis comparing modern sired Angus female and multi-generational sired Angus females for the trait of intramuscular fat**

<sup>a,b</sup> Superscripts indicate significance at  $P < 0.05$



**Figure 3.4: Means separation analysis comparing modern sired Angus female and multi-generational sired Angus females for the trait of ribeye area**

<sup>a</sup> Superscript indicates no significance at  $P < 0.05$



**Figure 3.5: Means separation analysis comparing modern sired Angus females and multi-generational sired Angus females for the trait of back fat thickness**

<sup>a,b</sup> Superscripts indicate significance at  $P < 0.05$

**Table 3.4: Level of significance, numbers of animals from each genotype, and S.E. for markers significantly associated with birth weight and weaning weight**

Trait (kg)	Gene	SNP ID	Allele <sup>3</sup>	Minor Genotype Frequency <sup>4</sup>	Het Genotype Frequency <sup>4</sup>	Major Genotype Frequency <sup>4</sup>	P-Value
BW <sup>1</sup>	LEPR	rs134375381	G/T	0	5	42	0.07**
BW	LEPR	rs135263435	G/A	0	3	44	0.03*
BW	LEPR	rs43348659	A/C	0	3	43	0.07**
WW <sup>2</sup>	DGAT1	rs136875432	A/G	2	11	34	0.10**
WW	DGAT1	rs135423283	G/T	2	11	34	0.10**
WW	DGAT1	rs132699547	C/G	2	11	34	0.10**

<sup>1</sup> Birth Weight

<sup>2</sup> Weaning Weight

<sup>3</sup> Representation of the minor allele is located on the left

<sup>4</sup> Number of animals inheriting each genotype

\* Superscripts differ P < 0.05 indicate significance

\*\* Superscripts differ P < 0.10 indicate statistical trend

than birth weights of animals that inherited the heterozygous genotype GA (Table 3.5).

A trend (P = 0.07) was observed for marker rs43348659 influencing birth weight performance. Animals inheriting the homozygous genotype AA for marker rs43348659 had birth weights that were higher than birth weights of animals that inherited the heterozygous genotype AC (Table 3.5). A trend (P = 0.07) was observed for marker rs134375381 influencing birth weight performance. Animals inheriting the homozygous genotype GG for marker rs134375381 had birth weights that were higher than birth weights of animals that inherited the heterozygous genotype GT (Table 3.5).

Three unique SNP located within DGAT1 were associated with weaning weight (rs136875432, rs135423283, and rs132699547) (Table 3.4). A trend (P = 0.10) was observed for marker rs136875432 influencing weaning weight performance. Animals inheriting the homozygous genotype GG for marker rs136875432 had weaning weights

**Table 3.5: Single nucleotide polymorphisms significantly associated with birth weight and weaning weight and least square means comparisons between reported genotypes**

Trait (kg)	Gene	SNP ID	Allele <sup>3</sup>	Minor Genotype Mean	Het Genotype Mean	Major Genotype Mean
BW <sup>1</sup>	LEPR	rs134375381	G/T		29.17 ± 4.00 <sup>a**</sup>	37.11 ± 1.06 <sup>b**</sup>
BW	LEPR	rs135263435	G/A		25.89 ± 4.68 <sup>a*</sup>	37.04 ± 1.00 <sup>b*</sup>
BW	LEPR	rs43348659	A/C		25.81 ± 4.71 <sup>a*</sup>	36.96 ± 1.01 <sup>b*</sup>
WW <sup>2</sup>	DGAT1	rs136875432	A/G	263.74 ± 33.62 <sup>a**</sup>	196.48 ± 12.52 <sup>b**</sup>	213.76 ± 6.73 <sup>ab</sup>
WW	DGAT1	rs135423283	G/T	263.74 ± 33.62 <sup>a**</sup>	196.48 ± 12.52 <sup>b**</sup>	213.76 ± 6.73 <sup>ab</sup>
WW	DGAT1	rs132699547	C/G	263.74 ± 33.62 <sup>a**</sup>	196.48 ± 12.52 <sup>b**</sup>	213.76 ± 6.73 <sup>ab</sup>

<sup>1</sup> Birth Weight

<sup>2</sup> Weaning Weight

<sup>3</sup> Representation of the minor allele is located on the left

\* Superscripts differ P < 0.05 indicate significance

\*\* Superscripts differ P < 0.10 indicate statistical trend

<sup>a,b</sup> Superscripts indicate difference within row

that were higher than weaning weights of animals that inherited the heterozygous genotype GA (Table 3.5). A trend (P = 0.10) was observed for marker rs135423283 influencing weaning weight performance. Animals inheriting the homozygous genotype TT for marker rs135423283 had weaning weights that were higher than weaning weights of animals that inherited the heterozygous genotype GT (Table 3.5). A trend (P = 0.10) was observed for marker rs132699547 influencing weaning weight performance. Animals inheriting the homozygous genotype GG for marker rs132699547 had weaning weights that were higher than weaning weights of animals that inherited the heterozygous genotype CG (Table 3.5). No SNP located on CAST were significantly associated with birth weight or weaning weight.

Two unique SNP located within LEPR were associated with back fat thickness (rs134375381 and rs135263435) (Table 3.6). A trend (P = 0.10) was observed for

**Table 3.6: Level of significance, numbers of animals from each genotype, and S.E. for markers significantly associated with back fat thickness**

Trait (cm)	Gene	SNP ID	Allele <sup>2</sup>	Minor Genotype Frequency <sup>3</sup>	Het Genotype Frequency <sup>3</sup>	Major Genotype Frequency <sup>3</sup>	P-Value*
BF <sup>1</sup>	LEPR	rs134375381	G/T	0	5	42	0.10*
BF <sup>1</sup>	LEPR	rs135263435	G/A	0	3	44	0.10*

<sup>1</sup> Back Fat Thickness

<sup>2</sup> Representation of the minor allele is located on the left

<sup>3</sup> Number of animals inheriting each genotype

\* Superscripts differ P < 0.10 indicate statistical trend

**Table 3.7: Single nucleotide polymorphisms significantly associated with back fat thickness and least square means comparisons between reported genotypes**

Trait (cm)	Gene	SNP ID	Allele <sup>2</sup>	Minor Genotype Mean	Het Genotype Mean	Major Genotype Mean
BF <sup>1</sup>	LEPR	rs134375381	G/T		0.8163 ± 0.33 <sup>a*</sup>	0.4178 ± 0.064 <sup>b*</sup>
BF	LEPR	rs135263435	G/A		0.8163 ± 0.33 <sup>a*</sup>	0.4178 ± 0.064 <sup>b*</sup>

<sup>1</sup> Back Fat Thickness

<sup>2</sup> Representation of the minor allele is located on the left

\* Superscripts differ P < 0.10 indicate statistical trend

<sup>a,b</sup> Superscripts indicate difference within row

marker rs134375381 influencing back fat thickness. Animals inheriting the heterozygous genotype GT for marker rs134375381 had back fat thicknesses that were larger than back fat thicknesses of animals that inherited the homozygous genotype GG (Table 3.7). A trend (P = 0.10) was observed for marker rs135263435 influencing back fat thickness. Animals inheriting the heterozygous genotype GA for marker rs135263435 had back fat thicknesses that were larger than back fat thickness of animals that inherited the homozygous genotype GG (Table 3.7). No selected SNP located on CAST or DGAT1 were significantly associated with back fat thickness. No selected SNP



located on LEPR, CAST, or DGAT1 were significantly associated with intramuscular fat or ribeye area in this population.

## **Discussion**

The hypothesis that multi-generational Angus females would have lower levels of performance for growth and production traits was validated in the current study.

However, a second hypothesis that multi-generation Angus would have more favorable carcass quality traits was disproven. The multi-generation sired Angus females had less desirable carcass traits when compared to modern sired Angus females for back fat thickness and intramuscular fat. This indicated a selection change for increased growth rate and increased carcass size in the modern sired Angus females. These trends have been reported in many previous studies (Enns et al., 2008; Parnell et al., 1997; McClure et al., 2010) and indicate that genetic selection for production traits have made progress over the generations. Furthermore, the current study validates that modern germplasm from modern animals is more beneficial to utilize for the improvement of modern herds due to the large amount of genetic improvement that has been made over the founding beef breed populations.

DGAT1 markers rs136875432, rs135423283, and rs132699547 were observed to be associated with weaning weight. Effects of DGAT1 markers on weaning weight have previously been reported (McClure et al., 2010). LEPR markers rs135263435 and rs134375381 were observed to be associated with birth weight and back fat thickness. Effects of LEPR markers on back fat thickness have previously been reported (Buchanan et al., 2002; Schenkel et al., 2005).

LEPR markers rs135263435 and rs134375381 were observed to be associated with birth weight and back fat thickness, indicating that a single marker could be associated with multiple traits. The current study also identified multiple markers significantly associated with economically important traits. However, prior to utilization, further experimentation must be conducted. Validation of SNP identified in the current study must be validated in other populations and other environments. Secondly, a greater number of SNP and a greater number of candidate genes must be evaluated in order to properly identify significant marker associations and identify SNP that account for the largest degree of variability for the trait of interest. Finally, proper utilization of SNP significantly associated with economically important traits is essential. Specifically, multiple trait interaction must be evaluated so that detrimental effects on other performance traits are minimized. Identification of all SNP associated with a trait and that SNPs potential trait interactions and evaluation of markers associated with multiple traits in putative QTL regions is necessary as selection for individual markers or traits can be antagonistic to other important traits.

Identification of all markers associated with birth weight, weaning weight, ribeye area, and back fat thickness on candidate genes or in coding regions of the genome would allow increased accuracy of selection for beef producers trying to incorporate increased performance, profit, and sustainability into their herds. The identification of the causative mutations accounting for the largest amount of variability for birth weight and weaning weight would allow for increased accuracy of selection in addition to focused genotyping of markers essential for selection for these specific traits. The main objective of the current study was to generate preliminary information about molecular

markers that could be utilized in marker assisted selection programs. The current study has identified three SNP on LEPR associated with birth weight and back fat thickness and three SNP on DGAT1 associated with weaning weight that with validation in other populations could prove a valuable asset to future MAS programs.

## **CHAPTER IV**

### **EVALUATION OF CANDIDATE GENES AND SUBSEQUENT EFFECTS ON FERTILITY AND MILK PRODUCTION TRAITS IN HOLSTEIN FEMALES**

#### **Introduction**

Dramatic improvement in milk production has been observed over the last five decades (Butler et al., 1989; Washburn et al., 2002). Subsequently, modern Holsteins produce more milk than those in previous generations. The lactation cycle is initiated and renewed by parturition, therefore an animal's ability to be reproductively efficient is dependant on that animal's ability to conceive and maintain pregnancy (Lucy, 2001).

Over the last several decades, selection in Holstein cattle has focused primarily on increasing milk production. However, during this time a dramatic decline in reproductive efficiency in Holstein females has been observed. Washburn and associates (2002) observed days open and services to conception in Holstein cattle between 1976 and 1999 increased to from 124 to 168 days and 1.91 to 2.94, respectively. Additionally, milk yield increased from 4753kg to 6375kg and fat yield increased from 228kg to 282kg.

Three known candidate genes leptin receptor LEPR, calpastatin CAST, and DGAT1 were selected based on their previously recorded associations with fertility and production traits in Holstein cattle (Ashwell et al., 2004; Garcia et al., 2006; Liefers et al., 2002). The objective of this study was to evaluate fertility traits and production traits in relation to services to conception, days open, and various milk production traits of Holstein females.

## Experimental Animals

One hundred and twenty-three Holsteins females born between 2004 and 2010 from the Louisiana State University Agricultural Center Research and Teaching Dairy Farm located in Baton Rouge, Louisiana were utilized. All Holstein females were maintained, managed, and evaluated at this location. Females were maintained on a dry mixed diet (Table 4.1). All lactating females were brought to the parlor twice daily for milking. Individual birth weights were recorded and milk production traits were collected for each animal.

**Table 4.1: Composition of the high lactation daily ration**

Ingredient	AM feeding	PM feeding
-----Kilograms of DM <sup>1</sup> per cow-----		
Alfalfa Hay	0.99	1.21
Dry Distillers Grains	1.11	1.36
Whole Cottonseed	1.11	1.36
Soy Hull Pellets	1.01	1.23
Soybean Meal 48	1.17	1.43
LSU Custom Mineral <sup>2</sup>	0.19	0.23
High Herd Mineral <sup>3</sup>	0.37	0.46
Molasses	0.40	0.49
Corn Silage	13.12	16.03
Total	21.29	26.02

<sup>1</sup> DM = Dry Matter

<sup>2</sup> LSU Custom Mineral is a balanced mineral formulated by LSU animal science nutritionists to satisfy the needs of the LSU dairy research and teaching herd in order to maximize milk production.

<sup>3</sup> High Herd Mineral is 5.5% Pro-lak, 19% Sodium bicarbonate, 43.5% Megalac, 24% Calcium carbonate, 8% Salt (as fed).

The CIDR Synch protocol (Accelerated Genetics, Baraboo, Wisconsin) was utilized prior to artificial insemination of frozen/thawed semen to synchronize Holstein females. Females that did not respond to the CIDR Synch protocol were identified and artificial insemination was repeated during the next observed estrus.

### **Blood Collection and DNA Extraction**

Blood samples were collected from all Holstein females via tail vein vena puncture. Blood was transferred into 20mL tubes and centrifuged at 4000rpm at 4°C for 20 minutes. White blood cell buffy coats were extracted and transferred to 250 $\mu$ L microcentrifuge tubes. Genomic DNA was isolated and purified from buffy coats using a previously described saturated salt procedure (Miller et al., 1988) (Appendix A). Two hundred microliter DNA working solutions were prepared by combining a mixture of rehydration buffer and 25ng/ $\mu$ L of DNA. Extracted DNA and working solutions were stored at -4°C.

### **SNP and Genotyping**

Previously reported single nucleotide polymorphisms (SNPs) on candidate genes LEPR, CAST, and DGAT1 were collected from the dbSNP website (<http://www.ncbi.nlm.nih.gov/projects/SNP/>). Due to its direct involvement with triglyceride synthesis, DGAT1 is considered a candidate gene for intramuscular fat deposition (Thaller et al., 2003). Previous studies have identified LEPR to be expressed in a variety of tissues including the liver and mammary glands (Bartha et al., 2005). In addition, previous research suggested that polymorphisms within LEPR might affect days to first service in dairy cattle (Clempson et al., 2011). Therefore, LEPR has emerged as a candidate gene in the evaluation of the regulation of reproduction

(Komisarek, 2010). Increased CAST gene expression has previously been described to decrease muscle protein degradation (Kubiak et al., 2008). Additionally, a SNP located on the CAST gene associated with daughter pregnancy rate (DPR) in Holstein cattle has been reported (Garcia et al., 2006). Therefore, CAST is considered a candidate gene for fertility and meat tenderness.

Single nucleotide polymorphisms were selected by identifying SNP that were evenly distributed over the entire length of each candidate gene. The justification for this selection method was to account for possible linkage associations with potential causative mutations located on the candidate genes. Selected SNP, forward and reverse primers and allele substitutions for LEPR, CAST, and DGAT1 are reported in Table 4.2, Table 4.3, and Table 4.4. IPLEX reactions for all genes and SNP were generated by Illumina Inc. (San Diego, California), SNP genotyping was conducted by NeoGen (Lincoln, Nebraska) utilizing Sequenom genotyping technology (Illumina Inc., San Diego, California).

### **Statistical Analysis**

The Holstein population was analyzed using the Mixed Model procedure of SAS (SAS Institute Inc., Cary, North Carolina). The model included fixed effects for sire, dam, and individual candidate gene SNP. Sire within birth year was also fitted as a random nested variable to account for potential confounding affects observed in the data. Dependent variables of average services to conception, average days open, milk yield, and protein yield were fit into the mixed model procedure in order to test for associations between SNP and the previously described traits. All statistical analyses were conducted using similar methodologies reported in previous studies

**Table 4.2: Single nucleotide polymorphism ID, allele substitution, and forward and reverse primer sequences utilized for amplification and visualization of genotypes for LEPR**

SNP ID	Allele Substitution	Forward Sequence	Reverse Sequence
rs135977111	A/G	CTTCTGTTCTCTTCCTTGCAAAACATGTAA	CAAGCTCCCTGGCAGTGGGATTTCCAGACA
rs133145962	A/G	TATCTTTGGCAGGAATGCAATCAAATGTGT	TTAATCAGTCATGTCTGACTCTTTGTGACC
rs43347905	A/G	TTTTCTCTGTGTCTTTTAAATGTCCTAACA	AATTTATTTATGTAATAACTGCATTTAACT
rs133109480	A/G	GGTTTACAGTCCATAGAGTCGCAAAGAGTC	GACATAACTGAGCTGCTAAGCTCAAGCACG
rs43347912	G/T	CTGGACGGCCAGGGGGTTCCCTGAACTAAT	TTTAAAGTCACCCTAGGAGTAGAACAGATA
rs43347914	A/G	AAGCTCTTCCCTGCCTTCCCTTTGATTTTT	CTCAGAAGCCATTTTCATAGTTCTAACATTG
rs43347917	A/T	TTTAACCAATCCATTGATTTTTAATGTATG	AGTGTAACATTTTCAAATATCAAGTGAAAA
rs136901371	C/T	GAGACAAGAGAGAAGAGTTCAGAATAAAAT	GGGCTTGATTAATGGAGCAGAATACTCAAA
rs43348634	A/G	CTAAGCTGCTAAGTCACTTCAGTCATGTCC	ACTCTGTGCGAACCCATAGATGGCCTCCCA
rs134577752	A/G	CTGAGCACACTTGTCTTACTTTACAAATAAC	CATGTTTCTTCTCTCAAAATTTTAGTTGGT
rs135915491	C/G	AGCAGCAAAGTGTTTGAAAAATTGAAGTA	ATAGTGATCCTCAAGATGTTTTGTGTGCAT
rs43348652	A/G	TCTCTGCCCAGTATTGTCTACCCCTGCTCT	TGAGGCAGGAACCTTTGTCTCACTCACCATT
rs134375381	G/T	CAAAGACAAGAGCCTTTTGCTTGGAGTAAT	AAGGTAGGAGAACATTCAGAGATGTGGTTA
rs135560721	C/T	TTTTGAGGAGATTCACTCATACTTCAATAT	GTACATTCAAGCTTTCATTCAAGATCAGCA
rs137541136	A/G	GCTATTTCAAATCCTAAAAGATGATGCTGT	AAAGTGTGGCACTCAATATGCCGGCAAATT
rs43348655	C/T	ACAGTCCATGGGGTCACAAAGAGTTGGACA	GACTGAGCAAATCACTTGGTGCTGCATAA
rs43348659	A/C	AAGAATAATATTTTAGAGAAATATTGATTC	CCTTGTCCTCGCCACACGACACTGGCACTG
rs137111668	C/G	CTCTCCTTATTAGAAAATTGTCATTTACTT	AATTGCATACCCACTTACTGTCAAGCAAAA
rs137842817	G/T	AAAGTTTAATTGGATGTTCTGATGGTTTTT	AAATCTGAGTAGTCATAACTCAAAGCTTAG
rs135263435	A/G	TACTAGAAGACACTGTGAAAATTCAACTTT	GGAATGACAGCTCCTCATTTTACTAGCTTT



**Table 4.3: Single nucleotide polymorphism 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
rs43529864	G/T	GTGGGAGCCAGCTCGGACGTACACGTGCTA	TCGGCGTGAGTTCAGGCTCACAAGTTGAAT
rs133108534	C/T	TTGTCCTATTTTTGATTGCAATGATTCTTT	TTCAGCCTCCTCAAGTCTGCCTTTGAATCC
rs134804900	A/G	TCTGAGTGAAATGTCTCCTACTTTAGGACC	GCATCCTGCACTTCCTGTCTTTGCTCCCGT
rs109727429	C/T	AGCTGGCTGACAGAGAGGAGAGCCAGGCTT	GCCCTGCTCCCGTGACATAAATCACTGCAG
rs133978255	G/T	CACAGAGTCGGACACGACTGAAGCGACTTA	CAGCAGCAGCATACTCTTAAGTAGTATCCA
rs135802918	G/T	AATTGGTCATTATATCACCACTGCCTAGAG	AGGACCAGGCTTCTAGCCAGGGTTGAGTAA
rs134187714	C/T	AATCCCATGGACAGAGGAGCCGCAAAGAGT	GGACAGGAATGAGCCACTTCACTTTCACTT
rs135598419	A/C	AGAGCGGTGCTTTGTATCTGTCTTTCAAGA	TGCAAAGTGTTTTCGTGAGATTTGACAGT
rs133440731	A/G	GGGTCACAAAGAGTCAGACATGTCTCAGCA	TCAGACAAACAGCAAGGGTGTTAATGCTTG
rs135336850	C/T	ATTCAGTGTTGGCTGAAATTCTACCGGTCT	GAGTCCAGAGTCCGCTCTCGCTCTCTTAGC
rs137673193	C/T	CAATTGCACCTGTGGAAGGACAGTCATTAA	ATATAGATAGTGAAAGTGAAAGTGTAGTT
rs110972443	A/C	CATCTGTTGATAGACTTATAGGTTGCTTCC	TGTGTTGGCTATTGTAAACAGTGCTTCAAT
rs134668965	G/T	TTATTGTTTTTCAGACTGTTGCTAGGATTAT	ATCAACCAGACACCAACAGCCATTTCTCTC
rs133997237	C/T	AATGAATAAAAGAGCACAGGGCAATCCGTT	ATGAGATGCATTTTATTTGGAAGAGGTGGA
rs133149410	A/G	TAATGTCTCTGCTTTTTAATACCAGGGAAT	TGTTAAATTTCTCTAGAAAGCTAGCAAAC
rs110647227	A/G	TCCTTAGGCATTCAAGAAAATCATGCTCAC	GCGGGTAGGGTAGCAGACYGTATTGTTGGT
rs109491082	G/T	TACAGAGATCGGGCTTCTGAGTCTCATGTT	TCCACCCGGTTTCCATTGCCAAGGACCAAG
rs111010631	C/T	ACACACTGAAGGAGCTTAATATATTGTTGC	TTATTAGAATTGAAGTGCAATAATGCATAT
rs133820366	A/C	AAGGCCTGCTGTCTCTCTTTCTTCCCAAC	CCACCACCACCGGTGCTGTTGAGAACGAAG
rs136073124	C/T	GCCCTGTGTTTGATTCTACTTTACAGTAAC	GAAGAGCTGGTTTGGATGAGGGAGACTCTG

**Table 4.4: Single nucleotide polymorphism ID, allele substitution, and forward and reverse primer sequences utilized for amplification and visualization of genotypes for DGAT1**

SNP ID	Allele Substitution	Forward Sequence	Reverse Sequence
rs134049142	A/G	GGCACCCCTGTATGATGAGGGGCATGTGCCA	AGGGTGCCTGTGGCGAGCTCCCCACCTTGC
rs135576599	A/G	CCCCAGGGGATTCATGCAGGGAGGCCGTAG	AGCAGGCAGGGCCAGATGCCCAGCAAGACC
rs109711965	G/T	TGCCTGCCCTTTGGTGTGGCAGCCCCCTTCA	GCCTCACCTCAGCCTTGGCGCCGGCAGCCT
rs134455341	A/G	GGAAAGGGAGTGGAGATGACCTTGAACACC	TGTCCTTTGCTTTTCTCGGGTCTCTGACCC
rs134374261	A/C	GCACAGCCGGGCGCGCAGCAGCTGTCAGCCC	CCTGCCGCCCCTGCAAGTCCTGTCTCCCCA
rs137617619	A/G	TGCCCGACTCCTGTGACCCCATGGATTGTA	CCCACCAAGCTCCTCTATCCATGGGATTTT
rs135048973	C/T	ATTGCCACCTAGGAAGCCCCCCCCCCCCACC	CCTTTGAATATTCTTGTCTCTTTTCCTTGT
rs136875432	A/G	TGCCCCCCTCCTCTTCGGGAGACCATGCAC	TTCTACGCAGCCTGGCACATCTGGCAGACA
rs132679620	A/G	TCCTGGGGCCTCGGGGGCAGAGTGTGTGTT	TGCAAAGACAAGGCCATCTGCCAGCAACCC
rs132778108	C/G	AGGAGCTGCAGCTTCGGCACCCCCCAACCC	CCCCCGCCACTCACCTCGGGTAGGTTCT
rs109701809	A/G	CTGTCTGCCCCGCGGGGTATGTGTATCCTG	TGTCGTGTCCCGGGTTTGCTTGGCCCCCTCC
rs134718967	C/G	GTGCTCCCTCAACCTCAGGGGCACTCGGGT	ACACCGGGCACAGTCAGGTTAGCAACCCCC
rs109663724	A/T	GTGCTGAACCACGCGCGTGGCGTGTACCAT	TCTCCATCCAGGGCCGCACCGTGTGTACAGG
rs135423283	G/T	GCTGCTGTGGGAGCAGAGAAGTCACTTCGG	TTCCTGTGAGGGTTTTTCCTCAGGGCCATG
rs132669273	C/T	CACGAATGTAAGTAGCCACACAGTCCAC	ATCTGGCTCCTCCCAAGACCTCCAGCATCT
rs109169510	A/T	GGCTAAGGGGATGTTCTGCCCAAAAAGGA	GCAGGCAGGGTCTGGTGGGACTTCCTAGTA
rs137584522	C/G	AGATGAACCGCTCGGCCGAGGGGGATCCCT	CCCCACCCCCACTGCGGTCCCGCCGGCTG
rs132699547	C/G	GGCCGCCCACCTATCGGGGCAGAGGCAGTA	CAGTGCCCCCATCCCTGGAGCAGGGTCAGG
rs134110051	A/G	ACGGCCGCTGGGCAGCAGGTTTCTTCTGCC	CGGTGGCACAGGCACCTGGGGTTGTGGTTG
rs135143198	C/T	GGGGCTCAGCTCACTGTCCGCTTGCTTCCT	CCCCAGCTGTTCTCACCAGCTCCAGGTG

(White et al., 2005). Single nucleotide polymorphisms with more than one genotype represented were included in the analysis. Any SNP with only one genotype was excluded. Each SNP was evaluated independently. Due to a limited sample population statistical significance was evaluated at ( $P < 0.05$ ) and statistical trend was evaluated at ( $P < 0.10$ ).

## **Results**

One unique SNP located within LEPR was associated with average services to conception (rs135560721) (Table 4.5). A trend ( $P = 0.06$ ) was observed for marker rs135560721 influencing average services to conception. Animals inheriting the homozygous genotype CC for marker rs135560721 had fewer average services to conception than animals that inherited the heterozygous genotype CT (Table 4.6). No selected SNP located on CAST or DGAT1 were significantly associated with average services to conception.

Two unique SNP located within CAST were associated with average days open (rs134804900 and rs137673193) (Table 4.5). A trend ( $P = 0.10$ ) was observed for marker rs134804900 influencing average days open. Animals inheriting the homozygous genotype AA for marker rs134804900 had fewer average days open than observed for marker rs137673193 influencing average days open. Animals inheriting the homozygous genotype TT for marker rs137673193 had fewer average days open than animals that inherited the homozygous genotype CC (Table 4.6). Animal birth year and sire significantly ( $P < 0.001$ ) affected average days open. No selected SNP located on LEPR or DGAT1 were significantly associated with average days open.

**Table 4.5: Level of significance, numbers of animals from each genotype, and S.E. for markers significantly associated with average services to conception and average days open**

Trait	Gene	SNP ID	Allele <sup>3</sup>	Minor Genotype Frequency <sup>4</sup>	Het Genotype Frequency <sup>4</sup>	Major Genotype Frequency <sup>4</sup>	P-Value <sup>*</sup>
STC <sup>1</sup>	LEPR	rs135560721	C/T	1	19	100	0.06 <sup>*</sup>
DO <sup>2</sup>	CAST	rs134804900	A/G	5	16	101	0.10 <sup>*</sup>
DO	CAST	rs137673193	T/C	3	0	119	0.08 <sup>*</sup>

<sup>1</sup> Services to Conception

<sup>2</sup> Days Open

<sup>3</sup> Representation of the minor allele is located on the left

<sup>4</sup> Number of animals inheriting each genotype

<sup>\*</sup> Superscripts differ P < 0.10 indicate statistical trend

**Table 4.6: Single nucleotide polymorphisms significantly associated with average services to conception and average days open and least square means comparisons between reported genotypes**

Trait	Gene	SNP ID	Allele <sup>3</sup>	Minor Genotype Mean	Het Genotype Mean	Major Genotype Mean
STC <sup>1</sup>	LEPR	rs135560721	C/T	2.01±0.53 <sup>ab</sup>	2.89±0.37 <sup>a*</sup>	2.07±0.13 <sup>b*</sup>
DO <sup>2</sup>	CAST	rs134804900	A/G	162.62±23.67 <sup>ab</sup>	127.66±13.48 <sup>a*</sup>	115.32±6.8 <sup>b*</sup>
DO	CAST	rs137673193	T/C	173.73±33.24 <sup>a*</sup>		116.75±6.66 <sup>b*</sup>

<sup>1</sup> Services to Conception

<sup>2</sup> Days Open

<sup>3</sup> Representation of the minor allele is located on the left

<sup>\*</sup> Superscripts differ P < 0.10 indicate statistical trend

<sup>a,b</sup> Superscripts indicate difference within row

Two unique SNP located on CAST were associated with average protein yield (rs133149410 and rs137673193) (Table 4.7). A trend (P = 0.10) was observed for marker rs133149410 influencing average protein yield. Animals inheriting the heterozygous genotype AG for marker rs133149410 had greater protein yield than animals that inherited the homozygous genotype AA (Table 4.8). A trend (P = 0.10) was

**Table 4.7: Level of significance, numbers of animals from each genotype, and S.E. for markers significantly associated with average protein yield and average milk yield**

Trait (kg)	Gene	SNP ID	Allele <sup>3</sup>	Minor Genotype Frequency <sup>4</sup>	Het Genotype Frequency <sup>4</sup>	Major Genotype Frequency <sup>4</sup>	P-Value
PY <sup>1</sup>	CAST	rs133149410	A/G	14	58	31	0.10 <sup>**</sup>
PY	CAST	rs137673193	T/C	3	0	119	0.10 <sup>**</sup>
PY	DGAT1	rs109663724	T/A	0	1	119	0.07 <sup>**</sup>
MY <sup>2</sup>	CAST	rs133149410	A/G	14	58	31	0.10 <sup>**</sup>
MY	DGAT1	rs109663724	T/A	0	1	119	0.09 <sup>**</sup>
MY	LEPR	rs137111668	C/G	8	0	113	0.04 <sup>*</sup>

<sup>1</sup> Average Protein Yield

<sup>2</sup> Average Milk Yield

<sup>3</sup> Representation of the minor allele is located on the left

<sup>4</sup> Number of animals inheriting each genotype

\* Superscripts differ P < 0.05 indicate statistical significance

\*\* Superscripts differ P < 0.10 indicate statistical trend

**Table 4.8: Single nucleotide polymorphisms significantly associated with average protein yield and average milk yield and least square means comparisons between reported genotypes**

Trait (kg)	Gene	SNP ID	Allele <sup>3</sup>	Minor Genotype Mean	Het Genotype Mean	Major Genotype Mean
PY <sup>1</sup>	CAST	rs133149410	A/G	257.72±22.48 <sup>ab</sup>	282.42±17.25 <sup>a**</sup>	247.44±17.38 <sup>b**</sup>
PY	CAST	rs137673193	T/C	327.11±45.21 <sup>a**</sup>		261.61±13.28 <sup>b**</sup>
PY	DGAT1	rs109663724	T/A		486.09±117.19 <sup>a**</sup>	262.65±13.65 <sup>b**</sup>
MY <sup>2</sup>	CAST	rs133149410	A/G	800.38±800.38 <sup>ab</sup>	594.96±594.96 <sup>a**</sup>	8013.6±624.59 <sup>b**</sup>
MY	DGAT1	rs109663724	T/A		15971.8±4169.0 <sup>a**</sup>	8496.4±441.75 <sup>b**</sup>
MY	LEPR	rs137111668	C/G		10307.3±971.95 <sup>a*</sup>	8314.1±422.84 <sup>b*</sup>

<sup>1</sup> Average Protein Yield

<sup>2</sup> Average Milk Yield

<sup>3</sup> Representation of the minor allele is located on the left

<sup>4</sup> Number of animals inheriting each genotype

\* Superscripts differ P < 0.05 indicate statistical significance

\*\* Superscripts differ P < 0.10 indicate statistical trend

observed for marker rs137673193 influencing average protein yield. Animals inheriting the homozygous genotype TT for marker rs137673193 had lower average protein yield than animals that inherited the homozygous genotype CC (Table 4.8). One unique SNP located on DGAT1 was associated with average protein yield (rs109663724) (Table 4.7). A trend ( $P = 0.07$ ) was observed for marker rs109663724 influencing average protein yield. The animal inheriting the heterozygous genotype TA for marker rs109663724 had greater average protein yield than animals that inherited the homozygous genotype TT (Table 4.8). Animal birth year significantly ( $P < 0.001$ ) affected average protein yield. No selected SNP located on LEPR were significantly associated with average protein yield.

One unique SNP located on CAST was associated with average milk yield (rs133149410) (Table 4.7). A trend ( $P = 0.10$ ) was observed for marker rs133149410 influencing average milk yield. Animals inheriting the homozygous genotype AA for marker rs133149410 had greater milk yield than animals that inherited the heterozygous genotype AG (Table 4.8). One unique SNP located on DGAT1 was associated with average milk yield (rs109663724) (Table 4.7). A trend ( $P = 0.09$ ) was observed for marker rs109663724 influencing average milk yield. The animal inheriting the heterozygous genotype TA for marker rs109663724 had greater average milk yield than animals that inherited the homozygous genotype TT (Table 4.8). One unique SNP located on LEPR was significantly ( $P = 0.04$ ) associated with average milk yield (rs137111668) (Table 4.7). The animals inheriting the homozygous genotype GG for marker rs137111668 had greater average milk yield than animals that inherited the

homozygous genotype CC (Table 4.8). The birth year of the animals was a significant ( $P < 0.001$ ) source of variation in the model when evaluating average milk yield.

## **Discussion**

The hypothesis that allelic variation between low producing Holstein females and high producing Holstein females would be observed for average services to conception, average days open, average milk production, and average protein production was validated in this study. The high protein producing Holstein females had longer average days open period than low protein producing Holstein females. This indicates that the high producing animals are also the less reproductively efficient animals. This trend has been reported in previous studies (Pryce et al., 2000; Veerkamp et al., 2003) and indicates that genetic selection in Holstein cattle has focused primarily on increasing production but has resulted in a dramatic decline in reproductive efficiency.

CAST markers rs134804900 and rs137673193 were observed to be associated with average days open but was not significant for the trait. Effects of CAST markers on longevity and fertility in dairy cattle have previously been reported (Garcia et al., 2006). LEPR marker rs135560721 was observed to be associated with average services to conception but was not significant for the trait. Effects of LEPR markers on fertility in dairy cattle have previously been reported (Almeida et al., 2003). The lack of significance for average services to conception and average days open indicates that the differences observed in these traits could be influenced by management decisions and environmental factors. DGAT1 marker rs109663724 was observed to be associated with average milk yield and average protein yield. Effects of DGAT1 markers on milk

production and milk composition in Holstein cattle have previously been reported (Grisart et al., 2002).

Sire significantly ( $P < 0.001$ ) affected average services to conception. This could be attributed to the selection process in the dairy industry being focused toward new unproven sires versus efficient sires. Animal birth year significantly ( $P < 0.001$ ) affected average days open, average protein yield, and milk yield. This factor could be attributed to changes in management techniques or environmental fluctuations.

The current study indicates that a single marker could be associated with multiple traits and identified multiple markers significantly associated with economically important traits. However, prior to utilization, further experimentation must be conducted. Validation of SNP identified in the current study must be validated in other populations and other environments. Secondly, a greater number of SNP and a greater number of candidate genes must be evaluated in order to properly identify significant marker associations and identify SNP that account for the largest degree of variability for the trait of interest. Finally, proper utilization of SNP significantly associated with economically important traits is essential. Specifically, multiple trait interaction must be evaluated so that detrimental effects on other performance traits are minimized. Identification of all SNP associated with a trait and that SNPs potential trait interactions and evaluation of markers associated with multiple traits in putative QTL regions is necessary as selection for individual markers or traits can be antagonistic to other important traits.

Identification of all markers associated with average services to conception, average days open, average protein yield, and average milk yield on candidate genes



or in coding regions of the genome would allow increased accuracy of selection for dairy producers trying to incorporate increased performance, profit, and sustainability into their herds. The identification of the causative mutations accounting for the largest amount of variability for average services to conception, average days open, average protein yield, and average milk yield would allow for increased accuracy of selection in addition to focused genotyping of markers essential for selection for these specific traits. The main objective of the current study was to generate preliminary information about molecular markers that could be utilized in marker assisted selection programs. The current study has identified one SNP on CAST associated with average days open and average protein yield. One SNP on DGAT1 and CAST, respectively, has been identified as being associated with average protein yield and average milk yield that with validation in other populations could prove a valuable asset to future MAS programs.

## **CHAPTER V SUMMARY**

The studies presented herein validated that multi-generational Angus females have lower levels of performance for growth and production traits and proved that multi-generational Angus have less favorable carcass quality traits. Increased calf birth weight and longer coat length decrease the multi-generational Angus females' longevity in the herd. Therefore, modern germplasm from modern animals is more valuable for production improvement in a modern production scheme than multi-generational germplasm.

The studies presented herein also validated that allelic variation between low producing Holstein females and high producing Holstein females was observed for average services to conception, average days open, average milk production, and average protein production. This indicated that Holstein females selected for increased production have lower reproductive efficiency. Sire and animal birth year significantly affected average days open, average protein yield, and milk yield. This factor could be attributed to changes in management techniques including duration of time spent in the milking parlor, artificial insemination protocols, dietary changes, or environmental fluctuations. Differences in production traits and fertility traits that exist in modern Holstein cattle have identified the Holstein breed as a potentially suitable population to utilize MAS.

By utilizing candidate genes associated with reproductive efficiency and carcass traits it was possible to evaluate linkage associations with potential causative mutations for multiple traits. Continued evaluation LEPR, CAST, DGAT1, and the markers

contained within them must be further evaluated prior to being implemented into a marker assisted selection program.

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## APPENDIX A: DNA EXTRACTION – SATURATED SALT PROCEDURE

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

**Day 1:** in 15ml centrifuge tube

**Add:** 10-12mL Lysis buffer (Appendix B) to 250 $\mu$ L white blood cell buffy coat; invert to mix

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

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

**Add:** 200 $\mu$ L 10%SDS and 60 $\mu$ L of 10mg/mL RNase A; invert to mix; incubate 1hr at 37°C with gentle shaking

**Add:** 25 $\mu$ L of 20mg/mL Proteinase K; invert to mix; incubate overnight at 37°C with gentle shaking

**Day 2:**

**Add:** 1mL saturated NaCl; shake vigorously for 15secs

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

**Add:** 10-12mL 100% cold ethanol; invert to mix

**Remove:** DNA with soft pipette; transfer to 1.5mL tube

**Spin:** at 10 setting for 10mins in refrigerated bench-top centrifuge; aspirate off ethanol

**Add:** 1mL 80% cold ethanol; vortex for 20secs; spin 5mins in refrigerated bench-top centrifuge; aspirate off ethanol

**Add:** 500 $\mu$ L 80% cold ethanol; vortex for 20secs; spin 5mins in refrigerated bench-top centrifuge; aspirate off ethanol

Leave tubes uncovered to allow pellet to dry overnight

**Add:** 350 $\mu$ L Rehydration buffer (Appendix B) to resuspend DNA

## **APPENDIX B: BUFFER SOLUTION LABORATORY PROTOCOL**

### **LYSIS BUFFER (1L):**

7.49g  $\text{NH}_4\text{Cl}$   
2.059g trisHCl

pH to 7.4

### **DIGESTION BUFFER (1L):**

1.211g trisHCl  
23.376g NaCl  
0.744g EDTA

pH to 8.0

### **REHYDRATION BUFFER (1L):**

1.21g trisHCl  
0.37g EDTA

## **VITA**

Rebecca Ann Hill was born in January 1986 to Dale and Mary Hill in Zanesville, Ohio. Rebecca attended Caldwell and Captain Shreve High Schools, graduating from Captain Shreve High School in Shreveport, Louisiana, in May of 2004. She received her Bachelor of Science degree in Animal, Dairy, and Poultry Sciences at Louisiana State University in May of 2010.

Under the direction of Dr. Matthew D. Garcia, Rebecca began her Master of Science degree at Louisiana State University in the fall of 2010. Upon successful completion of her Master of Science degree she intends to continue her studies at Louisiana State University, where she has been accepted to the Doctor of Philosophy program in the School of Veterinary Medicine's Comparative Biomedical Sciences department.