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DISTRIBUTIONS AND ASSOCIATIONS OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE LEPTIN GENE OF *BOS TAURUS* AND *BOS INDICUS* CATTLE

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 in Animal, Dairy and Poultry Sciences

By

Douglas H. Fischer, Jr.
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ABSTRACT

In recent years, the use of genetic markers has become more and more prevalent in beef breeding programs. This research focused on four previously identified single nucleotide polymorphisms in a leptin gene on chromosome 4 of beef cows. The SNP were E2FB, T945M, UA1, and UA2. Beef cows used in this research were maintained at the Louisiana State University AgCenter Central Research Station. Cows consisted of purebred *Bos tauras* and *Bos indicus* cattle as well as crossbreds.

The objectives were to estimate genotypic and allelic frequencies for each SNP and to determine the influence of cow breed type, cow age, and SNP genotypes on cow calving rate and date of calving in 2006, cow plasma leptin concentration, body condition score, and pregnancy status in September of 2006, and cow weight change from April to September in 2006.

Over all cows, each of the genetic markers showed polymorphism. Allelic frequency for T in these SNP was greater than 0.10. Within cow breed groups the trend for lower or higher frequencies of homozygous genotypes tended to be consistent. Genotypes TT in E2FB, TT in T945M, CC in UA1, and TT in UA2 had lower frequencies. Brahman cows were missing both CT and TT genotypes in UA2 and the UA2-TT genotype was not present in Braford, Romosinuano F1 and Brahman F1 breed groups.

Neither of the genetic marker genotypes influenced variation in plasma leptin level ($P > 0.05$). Several genetic markers had effects associated with cow traits that were of interest to this research. UA1 genotypes tended ($P=0.07$) to have an effect on calving rate. UA2 genotypes were associated ($P<0.05$) with calving date as well as

weight loss. Cow breed group influenced ($P < 0.05$) calving rate, Julian calving date, weight change, and palpation status. Six year and older cows had a larger plasma leptin level and two year old cows had lower body condition scores than other ages of cows. These results indicate that after adjusting for cow breed group and cow age, genetic markers UA1 and UA2 appear to be associated with several reproductive and weight change traits of beef cows.

CHAPTER 1

INTRODUCTION

The selection of livestock for superior genetic merit has been a challenging business for some time. The livestock industry has progressed over the past 50 years from using adjusted phenotypic values to compare prospective parents to the use of individual animal breeding values for economically important traits (Field, 2007). More recently, the identification of genetic markers that are closely linked to or a part of the DNA structure of a major gene for economically important traits has developed into an important area of research. Reproduction is considered one of the most economically important traits in beef cattle. The genetic variation associated with calving rate of beef cattle is considered to be relatively low (Guerra et al., 2006). Identification of genetic markers for major genes associated with measures of reproduction could aid in identification of young cows that might be more productive in the beef cattle industry.

The leptin gene is located on the fourth chromosome in the bovine genome (Barendse et al., 1994; Stone et al., 1996). The product of this gene is a 16 kDa protein that is primarily produced by white adipocytes (Houseknecht et al., 1998). Leptin has been implicated in the regulation of feed intake, energy expenditure, and whole-body energy balance in both humans and rodents (Houseknecht et al., 1998). Positive correlations between leptin serum levels and carcass traits such as marbling, 12^{th} rib back fat thickness, kidney pelvic heart fat, and quality grade in beef cattle were reported by Geary et al. (2003). Differences in muscling, fat thickness, and marbling were also found (Delavaud et al., 2002). An association between plasma leptin concentrations and

growth and carcass traits was demonstrated in six distinct genetic lines of swine (Berg et al., 2003). Almeida et al. (2003) reported that RFLP genetic marker genotypes in the leptin gene were significantly associated with calving date in a Brangus herd in Brazil. A weak correlation between leptin serum levels and cow body condition score (BCS) and a strong relationship between leptin serum levels and the size of adiposities has been described (Delavaud et al., 2002).

This research involves four previously identified single nucleotide polymorphisms (SNP) in the leptin gene: E2FB, T945M, UA1, and UA2. Most research with the leptin gene in beef cattle has been done with *Bos taurus* cattle. This research involves not only *Bos taurus* cattle, but also *Bos indicus* cattle and their crosses. The objectives of this research were:

1. To determine overall and breed specific frequencies of the E2FB, T945M, UA1, and UA2 genetic marker genotypes and alleles,
2. To determine the influence of leptin genetic marker genotypes on the circulating level of plasma leptin, and
3. To determine the influences of cow breed group, age of cow, and genetic marker genotypes on cow calving rate and calving date in the spring of 2006, cow body condition score and pregnancy status in September of 2006, and weight change from April to September in 2006.

CHAPTER 2

LITERATURE REVIEW

General Leptin Information

The leptin gene was discovered in 1994 in Jackson Laboratory C57BL/6J and SM/Ckc-+^{DAC} mice by Zhang et al. (1994) and Moon and Friedman (1997), respectively. Barendse et al. (1994) and Stone et al. (1996) reported that the bovine leptin gene is located on Chromosome (BTA) 4.

Zang et al. (1997) reported that the leptin gene has three exons. The coding region for the leptin gene is contained within exons 2 & 3. Exon 1 is non-translated. Part of exon 2 encodes a signaling peptide of 21 aa residues, which is not represented in mature leptin protein. The leptin gene has a 67% sequence identity among species. The leptin gene codes for a 3.5kb cDNA (Gong et al., 1996). The leptin product protein has an approximate weight of 16kD. Humans and mice have two forms of the leptin protein that differ by a single AA residue (Isse et al., 1995; Zhang et al., 1994). The two proteins are due to alternative splicing of the leptin mRNA (Isse et al., 1995; Oberkofler et al., 1997).

The product of the leptin gene is similar to the family of helical cytokines, including IL-2 and growth hormone (Houseknecht et al., 1998). The members of the interleukin 6 family of cytokines interact with their receptors through three different binding sites I, II, and III. Leptin contains a single disulfide bond that links two cysteines (Cys96 and Cys146) within the C and D helices, and this bond has been proven critical for the structural integrity and stability of leptin (Rock et al., 1996).

It was suggested that leptin's affect might be localized in domains between aa residues 106 and 140 (Grasso et al., 1997).Three structures within leptin that are important to the function of leptin are: N terminal (residuals 22-115) biological and receptor binding; C terminal (residuals 116-166) with loop structure, enhances activity of N terminal region; C terminal disulfide bond (Imagawa et al., 1998). Four mutations were found in the human leptin protein. The first of these was Arg41Glu, which produced no receptor binding function. The other three mutations (Asp61Asn, Ser148Asp, and Arg149Glu) had no affect on the functionality of leptin receptor binding (Verploegen et al., 1997).

Peelman et al. (2004) investigated several mutations in the leptin gene that resemble the binding sites I, II, and III. Mutations in binding site I at the C-terminus of helix D did not affect receptor binding, but showed a modest effect on signaling. Mutations in binding site II at the surface of helices A and C impaired receptor binding, but had only a limited effect on signaling. Site III mutations around the N-terminus of helix D prevented receptor activation without affecting binding to the receptor.

Genetic Polymorphisms

Several alleles of the BM 1500 microsatellite have been identified in the leptin gene (Buchanan et al., 2002). A single nucleotide polymorphism was discovered in the exon 2 region of the leptin gene. This SNP can be distinguished using the restriction enzyme Kpn2I, and was associated with carcass fat measures in a population of unrelated beef bulls. This SNP resulted in a substation of a cysteine (C) for a thymine (T). This substation causes the amino acid to change from an arginine to a cysteine. The researchers associated the T allele with fatter beef carcasses and the C allele to

leaner beef carcasses. The C and T alleles had a frequency of 0.54 and 0.46, respectively. Animals with the T allele were found to have higher levels of leptin mRNA, thus suggesting that the T allele imparts a partial loss of biological function.

It was suggested that the TT and TC genotypes of the Kpn2I SNP increased milk yield in dairy cows when compared to the CC genotypes. They also observed a non-significant decrease in fat percentage over lactation. There was also a non-significant increase in the protein yield associated with the T allele. The animals that were homozygous with the T allele had a significant increase in the SSC linear score (Buchanan et al., 2003).

Madeja et al. (2004) reported that the allelic distribution of the exon 2 SNP, know as Kpn2I, was 0.54 C and 0.46 T. They did not find any associations between the alleles of Kpn2I and production traits.

Schenkel et al. (2005) found that the E2FB (Kpn2I) SNP had allelic frequencies of the C and T alleles of 0.389 and .611, respectively. The C allele was associated with lower fat, more grade fat, and more lean. It was suggested that the T allele had a high degree of dominance due to the fact that the heterozygote had very similar values to the TT homozygote.

Choudhary et al. (2005) examined the allelic frequency of the Kpn2I SNP in *Bos indicus* and *Bos taurus* cattle as well as their crossbreeds. The frequencies for the C allele in the crossbreds, Holstein Friesian, and Jersey cattle were 0.82, 0.60, and 0.44, respectively. The T allele frequencies were 0.18, 0.40 and 0.56. It is thought that the low presence of the T allele in the crossbreds and Holstein Friesian cattle was due to the lack of T alleles in the Hariana line of cattle.

Kononoff et al. (2005) stated that the genotypic frequencies of Kpn2I were 24.9% CC, 50.5% CT, and 24.6% TT. The researchers also reported that 7.6% more animals with the CT genotype graded AAA or better over TT. The TT genotype animals also graded 7.1% higher than the CC animals. They also reported that the TT genotype animals had less red meat in their carcasses then those of the CT and CC genotypes.

Almeida et al. (2007) investigated the allelic frequencies for Kpn2I using Aberdeen Angus and Charolais cattle. It was determined that the number of C alleles was higher in Charolais cattle then in the Aberdeen Angus. The T allele is just the reverse of the C allele. They did not find an association between the Kpn2I SNP and weight gain.

In 2005, Nkrumah et al. identified three SNP'S in the bovine leptin gene: UASMS1 (UA1), UASMS2 (UA2), and UASMS3 (UA3). UA1 was not further researched due to the fact that its Mendelian inheritance and trait associations were identical to UA3. The UA2 SNP is a C to T substitution with allelic frequencies of 0.80 and 0.20 respectively. The UA3 SNP is a C to G substitution with allelic frequencies of 0.52 and 0.48 respectively. The UA2 T allele is significantly associated with an increase serum leptin concentration over that of the C allele. Animals with the TT genotype had a higher metabolic mid-point weight than those with the CC genotype, and this held true for the heterozygous as well. Average daily gain was also higher for animals with the TT and CT genotypes then those with the CC genotype. The TT animals also had a higher final weight than those of the CC and CT genotypes. The TT and CT animals consumed significantly more dry matter then the CC animals. The T allele was also associated with

large back fat thickness scores, more ultrasound marbling, and overall increase body fatness compared to the C allele.

Animals with the GG genotype tended to have higher residual feed intake, weighed heavier, grew faster, had a greater final body weight then those with the GC and CC genotypes. In addition, the GG animals also had higher backfat thicknesses than the animals of the GC and CC genotypes. There were no differences among the UA3 genotypes when examining serum leptin concentration, feed conversion ratio, marbling score and lean muscle area.

Canadian researchers (Schenkel et al., 2005) found that the UASMS1 (UA1) and UASMS3 (UA3) SNP's were completely linked, and therefore did not continue research with the UA3 SNP. No significant associations between UA2 and any meat or carcass quality traits. The UA1 SNP was significantly associated with fat level and tended to be related to grade fat and lean.

Liefers et al. (2004) identified the SNP T945M as a C to T substitution within the leptin receptor gene. They reported that the frequency of the CC and CT genotypes were 92.9% and 7.1% respectively. There were no TT genotypes reported. The T allele was associated with circulating leptin concentrations during late pregnancy, but not during lactation. All other associations were reported as combined affects with other previously identified SNP's.

Ehrhardt et al. (2000) reported that the mean serum leptin concentration for late lactation cows was 4ng/mL. It was later suggested that there is a positive correlation between plasma leptin concentrations and longissimus muscle fat thickness, marbling score, body fat, and BCS in cows during the last third of lactation. In 2002, it was

suggested that breed of cattle tended to have a small effect on the plasma leptin levels (Delavaud et al., 2002).

Liefers et al. (2003) suggested that dry matter intake, live weight, milk yield, and energy balance affect the serum leptin concentration. A higher DMI results in a higher leptin concentration. Positive energy balances are associated with higher leptin levels, the reciprocal is also proposed. While milk yield has a negative association with plasma leptin levels, higher milk yield results in lower leptin levels. It is also presented that leptin has a significant effect on first postpartum estrus as well as estrus expression. Cows did not seem to experience the first postpartum estrus until the threshold of 4 ng/mL was met. Cows that had higher leptin levels also seemed to have a more expressive estrus then those with low levels.

Research points to leptin as a possible trigger to the onset of puberty. Leptin has been shown to reverse inadequate gonadotropin production in mice and infertility in obese mice. It is believed that leptin acts as a metabolic gate signaling the body if it has adequate nutrition to support reproductive functions (Cunningham et al., 1999).

Endocrinology of Leptin

In animals in zero energy balance, leptin is highly correlated with adipocytes size in lean and obese mice (Houseknecht et al., 1996a-b). Food deprivation results in a rapid and drastic fall in leptin gene expression (Cusin et al., 1995; Frederich et al., 1995; Trayhurn et al., 1995; Kolaczynski et al., 1996). Subtle changes in the short term energy balance can have dramatic effects on the circulating level of leptin. A 10% reduction or increase in body weight with reduce leptin by 56% or increase leptin by 300% respectively (Considine et al., 1996; Kolaczynski et al., 1996). Leptin has shown a dose

dependent affect on food intake, loss of body weight, loss of fat depots, and an increase in energy metabolism (Pelleymounter et al., 1995; Levin et al., 1996).

Hormonal or metabolite control of leptin expression is suggested by coordinated alterations in leptin gene expression. One prime candidate for this controlling factor is insulin. Insulin plays a chronic role in the regulation of leptin levels (Houseknecht et al., 1998). Hyperinsulinemia increases leptin levels in rodents and humans (Cusin et al., 1995; Saladin et al., 1995; Utriainen et al., 1996; Vidal et al., 1996). In humans, leptin concentrations are pulsatile in nature (Licinio et al., 1997).

Glucocorticoids are other potent regulators of leptin expression (Houseknecht et al., 1998). It is suggested that leptin and cortisol interact in a negative feedback loop. Leptin directly inhibits cortisol synthesis by adrenal cells (Bornstein et al., 1997). Leptin is also down-regulated by adrenergic stimulation. This is indicated in studies using β3-adrenergic agonists, cold exposure, or dbcAMP (Gettys et al., 1996; Mantzoros et al., 1996; Slieker et al., 1996; Trayhurn et al., 1996). It has also been reported that leptin regulates growth hormone secretion (Carro et al., 1997).

The leptin promoter has a functional binding site for C/EBPα in the region -58 to -42 relative to the transcriptional start site (He et al., 1995; MacDougald et al., 1995; Hwang et al., 1996). The C/EBPα functions as a transcriptional activator, and plays a role in terminal adipocyte differentiation (Christy et al., 1989; Herrera et al., 1989; Umek et al., 1991; Freytag and Geddes, 1992). PPARγ also seems to regulate the transcription of leptin. Thiazolidinediones, pharmacological ligands for the PPARγ act to down regulate leptin mRNA (Lehmann et al., 1995; Saltiel and Olefsky, 1996).

Hollenberg et al. (1997) reported that PPARγ2 mediates down regulation of the leptin promoter by inhibiting C/EBPα.

Previous research has suggested a fat secreted factor that reports the status of body energy stores to the brain (Kennedy, 1953; Hervey, 1958; Hausberger, 1959; Coleman and Hummel, 1969). Research investigating the effects of exogenous leptin on ob/ob led to the first model that proposed leptin was the adipose factor. Banks et al. (1996) demonstrated that leptin, synthesized and secreted by white adipocytes, travels to the brain where it causes the release of factors that ultimately result in the reduction of food intake, increase energy expenditure, and increase physical activity. It is also suggested that leptin acts in a negative feedback loop to inhibit further expression.

Houseknecht et al. (1998) reported that research was underway to try to determine the targets of leptin action in the brain as well as their downstream targets. Neuropeptide Y is believed to be one of those major targets. NPY is a stimulator of food intake and inhibitor of brown fat thermogenesis (Billington et al., 1991). NPY also increases plasma insulin and corticosteroid levels (Billington et al., 1991; Dryden and Williams, 1996). The treatment of ob/ob mice with leptin induces a lowering of NPY before there is a change in body weight (Stephens et al., 1995; Schwartz et al., 1996). The relationship between NPY and leptin was further emphasized when it was reported that ob/ob mice that had the NPY knocked out had attenuated obese affects (Erickson et al., 1996). This indicates that NPY is not the only neuroendocrine target of leptin.

While the long form of the leptin receptor is found almost exclusively in the central system, many other tissues contain the short form (Tartaglia et al., 1995). Through attenuating insulin action, leptin has been implicated in causing peripheral

insulin resistance in HepG2 cells (Cohen et al., 1996), and rat1 fibroblasts (Kroder et al., 1996). Glucose uptake and glycogen synthesis were, however, increased with exposure to leptin (Berti et al., 1997). Peripheral insulin resistance might also be affected by leptin via pancreatic β cells (Kieffer et al., 1996). It is believed that leptin directly inhibits insulin secretion by the β cell due to changes in the ion channel function (Emilsson et al., 1997; Kieffer et al., 1997). New research has described a peripheral action on the secretion of cortisol by leptin (Bornstein et al., 1997).

The concept of leptin resistance comes from the fact that most of the obesity studies stem from organisms that exhibit hyperleptinemia. The most obvious cause of this resistance is a defect in the leptin receptor itself (Houseknecht et al., 1998). A defect in the leptin receptor might explain the resistance to leptin that is observed in the db/db mice. Defects in the leptin gene product might also have an affect on the resistance to leptin. Many of the cytokine proteins bind to binding proteins in plasma. These binding proteins may have significant affects on the bioactivity of the bound protein. Proteins that have a defective structure might have reduced bioactivity ability and, therefore, present a form of leptin resistance (Heaney and Golde, 1993; Bonner and Brody, 1995). It has been found that the majority of leptin circulates in this bound form, and that the amount of free leptin is positively correlated with increasing obesity and body-mass index (Sinha et al., 1996; Housknecht et al., 1996). It is also possible that the bound form of leptin is responsible for its ability to transverse the blood brain barrier (Housknecht et al., 1998). Another source of leptin resistance suggested is not due to physiological defects of leptin or its promoter, but due to the limitations of leptin itself. It has been proposed that the teleological role of leptin is to avoid death by

starvation, not to avoid obesity (Speigelman and Flier, 1996). It is proposed that leptin can be a key factor in survival in times of feast and fast. Ahima et al. (1996) supports this belief by demonstrating that exogenous leptin can partially reverse neuroendocrine adaptations of starved mice.

Inadequate nutrition delays or prevents the onset of puberty (Foster and Olster, 1985; Bronson, 1986; Aubert and Sizonenko, 1996). This nutritional inadequacy also interferes with normal cyclicity (Howland, 1971; Vigersky et al., 1977; Armstrong and Britt, 1987). Leptin receptors can be found in the ventromedial and arcuate regions of the hypothalamus. These receptors are anatomically positioned to regions associated with the control of appetite and reproductive neuroendocrine function (Dyer et al., 1997). Mice that are ob/ob do not produce a functional form of leptin (Hamann and Matthaei, 1996). Female ob/ob mice remain in a prepubertal state with depressed ovarian and uterine weights, sex steroid concentrations, and pituitary gonadotropin secretion. These depressed functions can be restored to normal levels by administration of leptin. The same holds true for effects of under nutrition on reproduction in non-obese animals (Houseknecht et al., 1998).

Leptin has been shown to advance puberty in non-obese mice, as well as reduce feed intake (Chehab et al., 1997). It has been suggested that young people have a resistance to leptin due to the elevated levels of leptin (Hassink et al., 1996). This resistance could serve to maintain a high level of food intake and growth and to prevent early onset puberty. This is consistent with studies done in young pigs by Matteri et al. (1997). Leptin receptor mRNA has been found in ventromedial and arcuate hypothalamic nuclei and in anterior pituitary tissue of sheep (Dyer et al., 1997). Leptin

receptor mRNA has been found in the uterus, hypothalamus and pituitary gland of rats, as well as in the testis and ovaries of humans and rats (Schwarz et al., 1996; Zamorano et al., 1997; Cioffi et al., 1996).

It is thus believed that leptin could act at multiple sites in the reproductive system. Leptin treatment has been shown to enhance gonadotropin secretion (Barash et al., 1996). Hypothalamic GnRH has been recognized as deficient in ob/ob mice (Swerdloff et al., 1978; Batt et al., 1982). There is evidence that leptin effects hormone production in the anterior pituitary gland and (or) hypothalamus (Yu et al., 1997).

Leptin is exclusively produced and secreted by adipocytes (Kline et al., 1997) and placenta (Gong et al., 1996). However, leptin receptors are found in most tissues. The helical structure of leptin implied that the leptin receptor would be similar in structure and function to the helical cytokine receptors (Houseknecht et al., 1998). The leptin receptor, OBR, was confirmed to be similar to the gp130 signal transduction arm of class I cytokine receptor family members including interleukin-6, granulocyte colony stimulating factor, and leukemia inhibitory factor (Tartaglia et al., 1995). There are six alternatively spliced forms with cytoplasmic domains of different length, known as OBRa, OBRb, OBRc, OBRd, OBRe, and OBRf (Tartaglia et al., 1995; Tartaglia, 1997).

The extracellular domain of the long and short forms includes two cytokine domains, each containing a single copy of the characteristic Trp-Ser-X-Trp-Ser motif and fibronectin type III domain (White and Tartaglia, 1996). Long form, OBRb, is prevalent in the hypothalamus (arcuate, lateral, ventromedial, dorsomedial nuclei), but not present in most other tissues (Mercer et al., 1996; Schwartz et al., 1996), while the short forms are ubiquitously expressed. The short forms of the leptin receptor are

expressed by several non-immune tissues and seem to mediate the transport and degradation of leptin (La Cava et al., 2004). The long form of the receptor is expressed in various regions of the brain and is thought to be responsible for the central action of leptin (Tartaglia et al., 1995; Glaum et al., 1996; Mercer et al., 1996; Elmquist et al., 1997). Long form OBR, is expressed by the hypothalamus in areas that are responsible for the secretion of neuropeptides and neurotransmitters that regulate appetite, body weight (Friedman et al., 1998; Tartaglia et al., 1995; Tartaglia, 1997), bone mass (Ducy et al., 2000). Long form OBRb is also expressed by endothelial cells, pancreatic β-cells, the ovary, $CD34^+$ haematopoietic bone-marrow precursors, monocytes/macrophages, and T and B cells (Friedman et al., 1998; Sierra-Honigmann et al., 1998; Park et al., 2001; Ducy et al., 2000; Lord et al., 1998; Sanchez-Margalet., 2003; Tartaglia et al., 1995; Tartaglia, 1997). The expression of OBRb by T and B cells is of interest as it indicates a possible role of leptin in immune-cell activation and signal transduction, and might unveil new effects of leptin on, as yet unexplored, immune cell functions (Matarese et al., 2002; Sanchez-Margalet et al., 2003; Banks et al., 2000; Bjorbaek et al., 2001; Sweeney, 2002).

All isoforms of the OBR bind leptin; however, the long form appears to be of prime importance in signal transduction (Lee et al., 1996). Some receptor forms may also be involved in the transport of leptin in blood and its crossing of the blood-brain barrier (Devos et al., 1996). Leptin signaling via the JAK-STAT pathway has been reasonably well documented and is largely associated with the OB Receptor long form (Houseknecht et al., 1998). A point mutation within the OBR gene of the db mouse generates a new splice donor site that dramatically reduces the expression of the long

isoform in db/db mice (Lee et al., 1996). In db/db mice mRNA for the long form of the receptor is abnormal and yields a receptor with a truncated intracellular domain that is unable to appropriately signal (Chen et al., 1996; Chua et al., 1996; Chilardi et al., 1996; Lee et al., 1996; Vaisse et al., 1996). This mutation renders the mice resistant to the weight-lowering effects of endogenous and exogenous leptin (Halaas et al., 1995).

In summary, Zang et al. (1997) reported that the leptin gene has three exons, of which only the exon 2 & 3 contained the coding regions. It has also been shown that the product of this gene is similar to the family of helical cytokines, which include IL-2 and growth hormone (Houseknecht et al., 1998). Buchanan et al. (2002) indentified a SNP in the leptin gene that can be distinguished using the restriction enzyme Kpn2I. This SNP has been associated with carcass fat measures in a population of unrelated beef bulls. Animals with the T allele had higher level of leptin mRNA. Buchanan et al. (2003) suggested that the TT and TC genotypes of the Kpn2I SNP increased milk yield in dairy cows when compared to the CC genotypes. The C allele has been associated with lower fat and great lean in feedlot cattle by Schenkel et al. (2005). Kononoff et al. (2005) reported that animals with the CT genotype graded AAA or better 7.6% more than those of the TT genotype. Almeida et al. (2007) did not however find any associations between Kpn2I genotypes and weight gain in Aberdeen, Angus, and Charolais cattle. In 2005 Nkrumah et al. indentified three SNP in the bovine leptin gene. These SNP were UA1, UA2, and UA3. UA1 was found to have Mendelian inheritance pattern and trait associations that were identical to UA3 and was not further studied. The UA2 SNP produced a C to T substitution while the UA3 SNP produced a C to G substitution. The UA2 T allele was significantly associated with an increased serum leptin concentration

over that of the C allele. ADG was found to be higher in animals with the TT and CT genotypes over that of those with the CC genotype. The TT animals were also shown to have a higher final weight than those of the CC and CT genotypes. The T allele was associated with larger back fat thickness scores, more ultrasound marbling, and an overall increase in body fatness compared to the C allele. For the UA3 SNP cattle with the GG genotype tended to have heavier weights, faster growth, and greater final body weight then those of the GC and CC genotypes. There were no differences among the UA3 genotypes when examining serum leptin concentration, feed conversion ratio, marbling score, and lean muscle area. Schenkel et al. (2005) found no significant associations between UA2 and any meat or carcass quality traits, UA1 was however associated with fat level and tended to have a relationship with grade fat and lean. In 2004, Liefers et al. indentified the SNP T945M as a C to T substitution within the leptin receptor gene. The T allele showed a significant association with circulating leptin concentration during late pregnancy, but not during lactation.

Ehrhardt et al. (2000) reported that the mean serum leptin concentration in lactating cows was 4 ng/ml. A positive correlation between plasma leptin concentrations and longissimus muscle fat thickness, marbling score, body fat, and BCS in cows during the last third of lactation was suggested. It was suggested by Delavaud et al. (2002), that the breed of cow tended to have a small effect on the plasma leptin levels. Positive energy balances are associated with higher leptin levels, and the reciprocal has been proposed. Cows did not seem to experience the first postpartum estrus until the leptin enzyme had reached a threshold of 4ng/ml. Cows that had higher leptin levels tended to have a more expressive estrus then those with lower leptin levels. Results from

research suggest that leptin might be a trigger to the onset of puberty. Inadequate gonadotropin production has been reversed by leptin (Cunningham et al., 1999).

CHAPTER 3

MATERIALS AND METHODS

Source and Management of Cattle

Cattle utilized in this study consisted of 318 beef cows maintained at the Louisiana State University Agriculture Center Central Research Station in Baton Rouge. They consisted of purebred Angus, Brahman, and Braford as well as first-crosses (F1) sired by Beefmaster, Bonsmara, Brahman, Brangus and Romosinuano bulls. First cross cows from Brahman sires were from Angus dams. The dams of other F1 crossbreds were either Angus or MARC3 (1/4 Angus- ¼ Hereford- ¼ Red Poll- ¼ Penzgauer) cows from the USDA Meat Animal Research Center in Clay Center, NE. The 318 cows were composed of 42 Angus, 32 Brahman, 28 Braford, and 53 Bonsmara-, 43 Beefmaster-, 42 Brangus-, 39 Brahman-, and 39 Romosinuano-sired first-cross cows. First-cross females sired by Beefmaster, Bonsmara, Brangus, and Romosinuano bull were purchased from the US Meat Animal Research Center when they were weaned in the fall of 2001 and 2002. Angus, Brahman, and Braford cows ranged in age from 2 to 12 years; whereas, all crossbred cows were 4 and 5 years old when blood samples and data were collected in 2006. Because only a few aged cows were involved, cows older than 6 years old were reclassified as 6 years old.

The breeding season for all cows except the purebred Brahman started April 15 and ended July 1. The breeding season for Brahman cows started June 1 and ended August 15. The calving season for Brahman cows is March, April, and May. The calving season for all other breed groups of cows started January 15 and ended April 15. The breeding season for Brahman cows is later than for other cows because Brahman

calves have a poor survival when born in the normally wet and cool conditions in February.

Brahman calves were weaned the second week of October. All other calves were weaned the second and third week of September. Cows were palpated for pregnancy when calves are weaned.

Plasma Leptin Concentrations and Genotyping

Blood samples were taken in September and October when cows were palpated for pregnancy with 7 ml vacuum-container tubes via vena puncture through the jugular vein. A sample of the whole blood from each cow was placed on a Whatman FTA card for genotyping of the E2FB, T945M, UA1, and UA2 SNP's by Igenity Inc.

The remaining whole blood was centrifuged at 2000 rpm and 4°C for 20 minutes. Plasma (1.25ml) was then drawn off and placed into a 1.25mL sample container, which was then stored at -80°C. Plasma leptin concentrations were determined by radioimmunoassay as described by Cartmill et al. (2003). The specificity for bovine plasma was previously confirmed by 1) parallelism of inhibition curves generated with high bovine plasma samples with those produced by the porcine leptin standard, 2) identification of a single peak of immunoreactivity in a pool of high-leptin plasma fractionated by gel filtration (Sephadex G-200; Sigma Chemical Co., St. Louis, MO) coincident with radioiodinated leptin, and 3) correlations of plasma leptin concentrations with measures of body adiposity in heifers and cows. Limit of detection and intra- and interassay CV averaged 0.1 ng/mL and 6 and 4%, respectively.

Statistical Analyses

Cow response traits in this study were plasma leptin concentration, calving rate in 2006 (0 or 1), Julian calving date in 2006 (day of year), cow body condition score (BCS; 3, 4, 5, 6, and 7) in September of 2006, weight change of cows from April to September of 2006, and palpation status (0 or 1) for cows calving in 2007. Means, standard deviations, and minimum and maximum values of responses are given in Table 1.

Sire of cow information was available on all cows except those in the Brahman-sired F1 group. Cows with known sire information (n=265) were sired by 115 bulls. Number of daughters per sire ranged from 1 to 8 and averaged 2.3 cows. Thirty-four sires had only one daughter in this data set. Because of the low number of cows per sire and because sire was confounded with several genetic marker genotypes, the decision was made to not include cow sire in any of the statistical analyses. Under conditions of more daughters per sire, removing the random sire of cow influence would account for some of the additive genetic variation and allow a cleaner evaluation of genetic markers.

Cows were assigned to 8 breed groups for these analyses. These were purebred Angus, Brahman and Braford, and Beefmaster F1, Bonsmara F1, Brangus F1, Romosinuano F1, and Brahman F1 groups.

Data were analyzed with SAS (SAS Inst. Inc., Cary, NC; Version 9.1.3). Proc Allele was used to obtain overall genotypic and allelic frequencies for each genetic marker. Distribution of genetic marker genotypes in each of the eight sire breed groups of cows was also determined by using Proc Sort and Proc Freq.

Model 1 was a simple least squares procedure using GLM where fixed sources of variation included cow breed group, cow age, and the four SNP genotypes. Response traits were those shown in Table 1. The model may be written as $Y_{ijklmn} = \mu + CBG_i + CA_j + E2FB_k + T945M_l + UA1_m + UA2_n + e$, where Y_{ijklmn} = the response trait, CBG_i = cow breed group, CA_j = cow age, $E2FB_k$, $T945M_l$, $UA1_m$, and $UA2_n$ = genetic markers in the leptin gene, and e = residual error.

Model 2 contained the same sources of variation, but the genetic marker genotypes were recoded as numerical values in order to predict the regression of the response variables on each of the genetic marker numerical values. For E2FB genotype CC the numerical value was 0, representing two C alleles. Likewise, for genotypes CT and TT the numerical values for the genotypes were 1 and 2, respectively, where the numerical values represent the number of C alleles. For T945M, UA1 and UA2 a similar recoding of genotypes was done. The regression coefficient for each of these genetic markers will give the change in response value for each C allele in the genetic marker. The model may be written as $Y_{ijk} = \mu + CBG_j + CAGE_j + B_{1.gm1} + B_{2.gm2} + B_{3.gm3} + B_{4.gm4} + e_{ijk}$ where Y_{ijk} = each response variable, μ = overall mean, CBG_j = cow breed group, $CAGE_j$ = cow age, $B_{1.gm1}$ = the regression coefficient for each response on the number of C alleles in the genetic marker and e_{ijk} = residual error.

Model 3 was a GLM analysis to determine if plasma leptin concentration was influenced by genotypes in SNP T945M in pregnant cows in this study. Proc Corr in SAS was used to obtain simple correlations among cow response traits over the entire data set.

Table 1. Means, standard deviations, and minimum and maximum values for cow traits.

Trait[1]	No	Mean	Standard deviation	Minimum	Maximum
Leptin	263	1.17	1.8	0.01	12.24
JBD	271	46.1	19.2	1	113
BCS	300	5.2	0.9	3	7
CR%	296	92.3	0.3	0	1
PREG%	300	84.0	0.4	0	1
WTCH	278	41.2	80.2	-338	285

[1]JCD = Julian calving date, BCS = cow body condition score, CR% = calving rate percent, PREG% = pregnancy percent for 2007 based on palpation, WTCH = weight change of cows from April to September of 2006, and Leptin = plasma leptin concentration.

CHAPTER 4

RESULTS AND DISCUSSION

Genotypic and Allelic Frequencies

Genotypic and allelic frequencies over all cows for the four genetic markers are given in Table 2. T945M and UA2 SNP's had less than 5% homozygous TT genotypes however allelic frequencies were greater than 0.10 for each SNP. Hardy Weinberg logic should not be considered in these data because of the diverse cow breed types involved and the mating systems used to generate them. Only the Angus, Brahman, and Braford breed groups represent purebreds and were not mated at random. Unrelated bulls from outside the purebred herds were used through AI or were purchased, most likely reducing the resemblance of random mating based on genetic markers.

F1 breed groups of cows were made with unrelated bulls selected by the breed associations for use by the USDA Meat Animal Research Center. Angus and MARC-3 cows that the bulls were mated to were produced at the MARC and their relationship is unknown. Therefore, genotypic and allelic frequencies for the F1 cows should be an averaging of the frequencies in the breeds represented in the crossbreds, based on their degree of inheritance, and would no be expected to be in genetic equilibrium.

Numbers of genotypes for each genetic marker by cow breed group are given in Table3. Specific genotypes for some genetic markers were not present in several breed groups of cattle. However, the trend for higher or lower numbers of a particular genotype tends to be consistent throughout the breed groups. For example, genotypes

TT in the E2FB genetic marker, TT in the T945M genetic marker, CC in the UA1 genetic marker, and TT in the UA2 genetic marker have lower frequencies in all breed groups.

Table2. Genotypic and allelic frequencies of the E2FB, T945M, UA1, and UA2 SNP's over all cows in the study.

Genetic Marker	Genotype	No	Genotype Frequency, %	Allele	No	Allele Frequency, %
E2FB	CC	120	37.74	C	387	60.85
	CT	147	46.23	T	249	39.15
	TT	51	16.04			
T945M	CC	252	80.00	C	566	89.84
	CT	62	19.68	T	64	10.16
	TT	1	00.32			
UA1	CC	64	20.51	C	248	39.74
	CT	120	38.46	T	376	60.26
	TT	128	41.03			
UA2	CC	177	55.66	C	481	75.63
	CT	127	39.94	T	155	24.37
	TT	14	4.40			

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TT in the E2FB genetic marker, TT in the T945M genetic marker, CC in the UA1 genetic marker, and TT in the UA2 genetic marker have lower frequencies in all breed groups. Some breed groups have higher frequencies for the alternative homozygote whereas other breed groups have higher frequencies for the heterozygote.

Only one cow was identified with a T945M-TT genotype in this sample of cows, that being in the Romosinuano F1 group. Purebred Brahman cows were missing both CT and TT genotypes for the UA2 genetic marker. The UA2-TT genotype also was not present in the Braford, Romosinuano F1 and Brahman F1 breed groups.

The allelic frequencies for this sample of cattle are generally similar to frequencies found by others. The frequency for the E2FB genotypes from this sample of cattle resembled the frequencies reported by Choudhary et al. in 2005. Most of the other papers reported frequencies near 55% for the C allele and 45% for the T allele. Genotypic frequencies for the T945M SNP are similar to the frequencies reported by Liefers et al. (2004) who did not detect a TT genotype in 323 Holstein cows.

Small differences in the genotypic frequencies are possibly due to the difference in the genetic makeup of the herds used in previous research and the cattle used in this research. The allelic frequencies for the UA2 genetic marker in this sample of cattle are slightly different form frequencies reported earlier by Nkrumah et al. (2005). The frequencies for UA1 alleles reported by Schenkel et al. (2005) are similar to frequencies in this sample of cows.

Cow Response Traits

Significance levels, least squares means, and standard errors for cow response traits due to the four genetic markers are given in Table 4. Genetic marker E2FB did not

significantly influence variation in any of the cow response traits (P>0.05), however the probability level of P = 0.057 for cow Julian calving date suggests E2FB genotypes tend to be associated with this trait. SNP E2FB genotype TT had a least squares mean suggesting at least 10 days earlier calving than genotypes CC and CT.

Genetic marker T945M was limited to two genotypes (The one TT genotype observation was changed to CT for statistical analyses.) and did not significantly influence variation in any of the cow response traits.

Genetic marker UA1 significantly influenced variation (P<0.05) in calving rate in 2006 and P = 0.077 for Julian calving date in 2006. Genotype CC cows had a higher calving rate least squares mean (100.0±6.4) than genotypes CT and TT (85.8±5.5 and 85.9±6.0, respectively). Cows with the UA1 genotype CT had a slightly earlier calving date than UA1 genotypes CC and TT. Variation in cow Julian calving date, calving rate in 2006 and weight change from April to September was significantly influenced (P < 0.05) by genotypes of UA2. Cows with genotype TT calved at least 10 d earlier than cows with genotypes CC or CT. Cows with the heterozygous genotype CT had a higher calving rate in 2006 than cows with CC or TT genotypes (98.2±4.1 vs 85.0±3.4 and 90.3±8.4 %, respectively). Cows with the UA2-TT genotype lost weight (-42.4±27.0 lb) from April to September whereas cows with genotypes CC and CT gained weight (41.1±10.5 and 27.3±12.9 lb, respectively).

Table 3. Distribution of genetic marker genotypes by cow breed group.

Cow breed type	E2FB CC	E2FB CT	E2FB TT	T945M CC	T945M CT	T945M TT
Angus	13	18	7	38	--	--
Brahman	22	6	2	13	17	--
Braford	14	12	2	20	6	--
Beefmaster F1	18	29	6	48	5	--
Bonsmara F1	12	20	11	37	6	--
Brangus F1	9	21	12	40	1	--
Romosinuano F1	14	19	5	22	15	1
Brahman F1	14	18	6	28	10	--

Cow breed type	UA1 CC	UA1 CT	UA1 TT	UA2 CC	UA2 CT	UA2 TT
Angus	7	17	14	16	15	7
Brahman	3	2	24	29	--	--
Braford	3	6	14	8	15	--
Beefmaster F1	8	24	21	28	23	2
Bonsmara F1	11	19	13	21	20	2
Brangus F1	12	20	9	22	16	3
Romosinuano F1	10	18	10	21	17	--
Brahman F1	9	11	18	22	16	--

Table 4. Least squares means (±SE) and significance level for cow plasma enzyme, cow Julian calving date, calving rate in 2006, cow body condition in September 2006, cow body weight change April to September 2006, and September cow palpation rate for 2007 calves due to genetic markers.

Genetic marker and genotype	Leptin	Cow JCD	CR in 2006	BCS	Weight change	Palpation status
E2FB	0.828	0.057	0.318	0.817	0.488	0.916
CC	1.35±0.45	47.3±4.0	91.6±6.2	5.1±0.2	14.5±19.0	84.4±8.2
CT	1.66±0.34	46.8±2.9	95.5±4.7	5.0±0.2	-2.9±14.7	80.8±6.3
TT	1.58±0.50	36.7±4.5	86.4±6.8	5.0±0.2	14.9±21.7	81.6±9.2
T945M	0.219	0.265	0.369	0.883	0.234	0.420
CC	1.72±0.29	45.1±2.7	89.3±4.1	5.0±0.1	16.3±13.0	80.0±5.5
CT	1.35±0.35	42.2±3.1	93.1±4.8	5.1±0.2	1.1±15.2	84.5±6.6
UA1	0.913	0.077	0.038*	0.589	0.331	0.858
CC	1.39±0.45	48.4±4.1	100±6.4	5.0±0.2	-5.3±19.9	83.9±8.6
CT	1.56±0.40	39.7±3.6	85.8±5.5	5.2±0.2	21.9±17.2	84.1±7.4
TT	1.65±0.44	42.7±3.8	85.9±6.0	5.0±0.2	9.4±18.6	78.8±7.9
UA2	0.703	0.042*	0.004**	0.357	0.012*	0.482
CC	1.32±0.24	49.6±2.1	85.0±3.4	5.1±0.1	41.1±10.5	85.4±4.4
CT	1.39±0.29	45.7±2.6	98.2±4.1	4.9±0.1	27.3±12.9	87.0±5.5
TT	1.88±0.64	35.5±5.5	90.3±8.4	5.1±0.3	-42.4±27.0	74.3±11.3

*P < 0.05; **P < 0.01

Analyses from Model 2 had similar significance levels as Model 1. Regression of the response traits on number of C alleles for E2FB suggested a slight association (P = 0.062). The regression of cow Julian calving date on number of C alleles in E2FB was 5.6±3.0 d. Calving date was delayed about 5.6 days for each C allele in the E2FB genetic marker. The regression of all response traits on number of C alleles in T945M gave non-significant results (P > 0.10).

The regression of response traits on number of C alleles for genetic marker UA1 approached significance (P = 0.072) for calving rate of cows in 2006. Calving rate increased 8.4±4.6 % for each C allele present in UA1. For UA2, the regression of response traits on number of C alleles gave significant regression coefficients for cow Julian calving date (P < 0.035), calving rate in 2006, and cow weight change from April to September. Cow Julian calving date was delayed 4.7±2.2 d for each C allele in UA2. Calving rate in 2006 was decreased 9.0±3.4 % for each C allele in UA2. Cows gained 27.6±11.1 lb from April to September for each C allele in the UA2 genetic marker.

Significance levels, least squares means, and standard errors for cow response traits due to cow breed group and cow age are given in Table 5. Cow breed group significantly influenced variation (P < 0.05) in cow Julian calving date, calving rate in 2006, cow weight change from April to September, and in palpation status for cows calving in 2007. Cow age significantly influenced variation (P < 0.05) in plasma leptin concentration and cow body condition score in 2006.

For plasma leptin concentration, Angus cows had higher levels than Braford cows (P < 0.05). No other differences in plasma leptin concentration were observed

among cow breed types. Brahman calves were born later ($P < 0.01$) than other calves because the breeding season for Brahman cows started about 45 days later. Angus calves were born earlier ($P < 0.05$) than calves from Beefmaster F1, Bonsmara F1, Romosinuano F1 and Brahman F1 cows.

Calving rate for Brahman cows shown in Table 5 was 100±7.6 %. This calving rate mean is due to combining all Brahman cows that had calved into a single herd and this herd was sampled in the fall of 2006 when calves were weaned. Cows that did not calve in 2006 were not sampled or included in the analyses. Romosinuano F1 cows had a lower calving rate in 2006 than other F1 cows ($P < 0.05$). Bonsmara F1 cows gained more weight from April to September than Brahman cows as well as all F1 cows ($P < 0.05$). Palpation scores for 2007 calves were less for Angus (61.0±6.9 %) and Brahman (55.1±9.9 %) cows than for Barford cows (88.7±8.6 %) and all F1 cows ($P < 0.05$). No differences among F1 cows were found for palpation status.

Table 5. Least squares means (±SE) and significance level for cow plasma enzyme, cow Julian calving date, calving rate in 2006, cow body condition in September 2006, cow body weight change April to September 2006, and September cow palpation rate for 2007 calves due to cow breed group (CBG) and cow age.

Factor	Leptin	Cow JCD	CR in 2006	BCS	Weight change	Palpation status
CBG[1]	0.10	<.001**	0.017*	0.065	0.028*	<.001**
A	2.08±0.41	30.8±3.3	82.6±5.1	4.6±0.2	28.4±19.1	61.0±6.9
B	1.93±0.53	76.9±4.8	100±7.6	5.4±0.3	-15.7±22.8	55.1±9.9
BO	0.79±0.45	38.5±4.1	92.7±6.3	5.1±0.2	64.7±19.9	88.7±8.6
BM F1	1.11±0.41	40.8±3.8	90.0±5.8	5.1±0.2	-7.2±18.5	90.0±7.8
BO F1	1.33±0.42	40.9±3.6	93.2±5.8	5.2±0.2	-2.3±18.4	85.5±7.8
BR F1	1.62±0.43	37.2±3.8	77.8±5.8	4.9±0.2	9.1±18.7	94.4±7.9
RO F1	1.80±0.44	41.5±3.9	91.9±6.2	4.9±0.2	14.1±19.6	95.2±8.3
B F1	1.62±0.47	42.3±4.1	93.9±6.4	5.3±0.2	-21.6±20.7	88.4±8.7
Cow age	0.008**	0.102	0.063	0.001**	0.325	0.448
2	1.55±0.56	52.0±5.4	81.6±7.7	4.3±0.3	-23.7±26.5	87.1±10.4
3	1.21±0.62	46.3±5.7	80.9±8.7	5.5±0.3	15.3±26.9	86.0±11.7
4	0.93±0.30	41.7±2.5	98.5±3.9	5.1±0.1	27.5±12.7	84.3±5.3
5	1.18±0.31	41.8±2.5	94.3±4.0	5.4±0.1	10.9±13.0	75.9±5.4
6	2.79±0.49	36.2±4.5	100±7.1	5.1±0.2	13.3±23.1	78.1±9.6

* P < 0.05; **P < 0.01

[1] Cow breed groups: A=Angus, B=Brahman, BO=Braford, BM F1=Beefmaster F1, BO F1=Bonsmara F1, BR F1=Brangus F1, RO F1=Romosinuano F1, and B F1 = Brahman F1.

Cows 6 years and older had higher plasma leptin levels (2.79±0.49) than cows of all other ages ($P < 0.05$). The next highest plasma leptin level was 1.55±0.56 in 2 year old cows. Two year old cows also had the lowest body condition score among all cow ages. Their least squares mean for body condition score was 4.3±0.3 compared to the next lowest score of 5.1±0.1 in 4 year old cows and 5.1±0.2 in 6 + year old cows. It is not uncommon for young cows to have lower body condition scores when they wean their first calves.

T945M genotypes in pregnant cows were not associated with plasma leptin concentration. Cows in this study were in the second trimester of pregnancy when blood samples were collected whereas Leifers et al. (2004) sampled Holstein cows late in the third trimester of pregnancy.

Discussion

Of interest in this research was the genotypic and allelic frequencies of four single nucleotide polymorphisms (SNP) measured in a diverse group of beef cows. In addition, we wanted to know if any of the leptin SNP influenced variation in cow plasma leptin concentration, reproductive, body condition and weight change traits.

Plasma leptin concentration was determined from blood samples taken in September of 2006, when the cows were in the second trimester of pregnancy. Neither of the SNP influenced plasma leptin concentration in these cattle, but older cows had higher plasma leptin levels than 3, 4, and 5 year old cows. Angus, Brahman and Romosinuano F1 cows tended to have higher levels. Ciccioli et al. (2003) reported that mature Angus x Hereford cows on a higher feeding regime post calving gained more weight and had higher plasma leptin levels than cows on a lower feeding regime.

However, no differences in plasma leptin were detected prior or after the first postpartum estrus. Obeidat et al. (2002) reported that Brahman cows had higher serum leptin levels in September at late lactation than Angus cows when grazing the Chihuahuan Desert in New Mexico. This would have been in the middle trimester of pregnancy. We found no difference between Angus and Brahman cows at this stage of pregnancy.

Serum concentrations of leptin were positively correlated with measures of body fat across Angus, Brahman, and Brangus yearling heifers at the start of their first breeding season (Lopez et al., 2006). However, serum leptin concentration was not related to body weight or pregnancy rate at the end of the breeding season.

Leifers et al. (2004) reported that the leptin genetic marker T945M was associated with serum leptin concentration in late pregnancy of Holstein cows, but not during lactation. Kadokawa et al. (2006) reported that for Holstein cows in negative energy balance and mobilizing fat from adipose tissues, levels of serum leptin and LH are low, possibly resulting in delayed expression of estrus. Similar results with Holstein cows were reported by Leifers et al. (2003). Sampling cows in this study in September, at the end of lactation, and in the middle trimester of pregnancy does not appear to have been the best time in order to associate leptin levels with leptin SNP. Plasma leptin levels found in this study appear similar to others who measured leptin concentration at times different from late pregnancy or during the postpartum period.

Geary et al. (2003) and Nkruman et al. (2007) reported the association between serum leptin concentration and carcass traits in beef cattle. Blood samples were taken a few days before slaughter in both papers. Positive correlations existed between leptin

concentration and most measures of fatness, including backfat thickness and marbling. It seems to be clear that when cattle, steers or cows, are on a positive energy balance, leptin is correlated positively with measures of fatness. In this study the correlation between plasma leptin level and weight change from April to September was only 0.05 (P=0.39), indicating no association. Plasma leptin level also was not correlated with body condition score at the time blood samples were taken (r = - 0.01) even though body condition scores ranged from 3 to 7.

Both UA1 and UA2 genotypes influenced variation in calving rate in 2006. It is not clear if this association is due to different degrees of fatness because body condition scores were influenced by these markers. A small positive correlation (r = 0.16; P < 0.01) was found between weight change from April to September and palpation status for pregnancy, indicating that cows that gained more weight had a higher expectation for pregnancy.

CHAPTER 5

CONCLUSIONS

Frequencies of E2FB, T945M, UA1, and UA2 genetic marker genotypes in this sample of cows were generally similar to frequencies reported in the literature. Only one cow had the TT genotype in genetic marker T945M and Angus cows had only the CC genotype for T945M. Brahman cows had only the CC genotype for genetic marker UA2. T945M and UA2 genetic markers had relatively low frequencies for the T allele. Hardy Weinberg equilibrium was not expected as the cows that were sampled in this study were not from random mating populations.

Because the least squares model used to analyze the response traits included fixed effects cow breed group, cow age, and the four genetic markers, significance for any of the independent variables would mean that factor had a significant influence after the response trait was adjusted for the other fixed effects. Including only the genetic markers in the analyses could give significance for a response that was partially due to either cow breed group or cow age.

Genetic markers E2FB, UA1 and UA2 appear to have important effects on variation in cow Julian calving date and calving rate. The T allele in E2FB, UA1 and UA2 appears to code for earlier calving. The C allele in UA1 and T allele in UA2 appear to code for higher calving rate.

Plasma leptin concentration in mid gestation pregnant cows was not influenced by genotypes in the T945M genetic marker.

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VITA

Douglas H. Fischer, Jr., is the first son of Douglas H. and Sherry O. Fischer. Douglas attended high school at Pearl River High School in Pearl River, Louisiana. While in high school, Douglas took four years of agricultural classes where his interest in animal science was nurtured and grew. Douglas graduated with honors from Pearl River High in 2001.

In the fall of 2001, Douglas was accepted to Louisiana State University. At Louisiana State University, Douglas majored in animal sciences focusing in animal dairy and poultry sciences. While attending class at Louisiana State University, Douglas took classes taught by Dr. Donald Franke. In these classes, Douglas's interest in quantitative genetics grew. Douglas graduated from Louisiana State University in December 2005 with a Bachelor of Science degree.

Prior to graduation from Louisiana State University, Douglas decided to attend graduate school at Louisiana State University. Douglas was accepted and began studying quantitative genetics under Dr. Donald Franke in January of 2006. On December 22, 2006, Douglas married his longtime fiancé, Dora Louise Phelps. Douglas and Dora welcomed their first child, Christina Lillian Fischer, on March 15, 2007. Upon completion of his Master of Science degree, Douglas intends to attend Texas Agricultural and Mechanical University in College Station, Texas. At Texas Agricultural and Mechanical University, Douglas intends to pursue a Doctor of Philosophy in molecular genetics.