DNA MARKER EVALUATION OF BEEF CALVES SIRED BY HIGH AND LOW MARBLING EPD ANGUS BULLS

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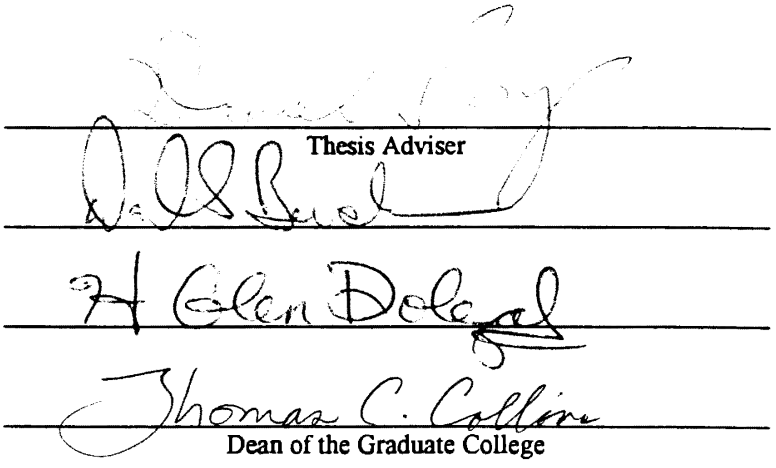

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NOMENCLATURE

AFAT	Adjusted Preliminary Yield Grade
AWW	Adjusted Weaning Weight
B-Lac	Beta-Lactoglobulin
H-FABP	Heart-Fatty Acid Binding Protein
HCW	Hot Carcass Weight
K-Cas	Kappa-Casein
LPL	Lipoprotein Lipase
MARB	Marbling Score
NTEB	Ninth-Tenth-Eleventh Bone Weight
NTEBN	Ninth-Tenth-Eleventh Rib Bone %
NTEEYE	Ninth-Tenth-Eleventh Longissimus Dorsi Weight
NTEFAT	Ninth-Tenth-Eleventh Rib Fat %
NTELEN	Ninth-Tenth-Eleventh Rib Lean %
NTERIB	Ninth-Tenth-Eleventh Rib Section
NTESM	Ninth-Tenth-Eleventh Seam Fat Weight
NTESC	Ninth-Tenth-Eleventh Subcutaneous Fat Weight
Pit-1	Pituitary Transcription Factor-1
REA	Rib-Eye Area
RIB	Rib Section Weight

CHAPTER I

INTRODUCTION

The desire to understand the genetic mechanisms behind the differences in body composition and marbling score observed between different breeds and within breeds of cattle is important for many reasons. The health conscious consumers' demand for a lean meat product with desirable palatability is probably the most obvious. The goal of livestock production has always been to produce a product that is desirable to the consumer. This is the only way for livestock production to be a profitable enterprise. To date, the most reliable measurement that is positively correlated with overall palatability is the amount of intramuscular adipose tissue present in the longissimus dorsi muscle (Jennings et al., 1978; Tatum et al., 1982; Savell et al., 1987; Jeremiah et al., 1992). Therefore, by understanding the genetic reasons for differences in marbling score and body composition seen in beef animals, the livestock industry may be able to produce a more consistent market animal in terms of carcass characteristics. The use of molecular genetic techniques may allow us to study genetic differences between beef cattle. If differences within the DNA sequence are found between animals then DNA tests may become possible to assist selection for superior carcass traits. This could lead to more accurate selection and shorter generation intervals to yield a faster rate of genetic improvement for increased marbling scores and superior carcass qualities. In summary, the understanding of the genetic mechanisms behind marbling and other carcass characteristics could yield a more consistent beef product in terms of palatability and yield of lean meat, and therefore provide the type of product desired by the consumers.

In addition to these uses for livestock selection, the identification of polymorphic genes or molecular markers in the bovine genome could be used to help identify the

chromosomal location of these genes or markers. This approach would help to expand on the existing bovine genome map (Barendse et al., 1994; Bishop et al., 1994) and further the knowledge about the relationship between genes and economically important traits in the bovine genome. Any markers or genes identified within this research project could become potentially useful markers for other important livestock traits. This means that any genes evaluated for their affects on marbling and carcass quality could be associated with other production traits such as yearling weight and average daily gain.

The goals of this research project are several fold. The primary goal is to attempt to identify markers or genes with an influence on carcass characteristics such as marbling and carcass fat as well as production traits such as birth weight and weaning weight in beef cattle. This goal would help the beef industry to identify animals with superior carcass qualities, and enhance the industry's ability to produce a desirable consumer product. Any polymorphic regions identified will be analyzed in a segregating population to test for significant genotype effects on carcass and production traits of interest. Secondary goals include the identification of chromosomal locations for any polymorphic regions identified that have not already been mapped in the bovine genome. The number of markers published with known chromosomal location for the bovine genome is still limited. However, large advances are being made in this area. In addition, the mapping of genes to their respective bovine chromosome may allow for an increased understanding of what regions of chromosomes are homologous between different species. Sections of bovine chromosome 1 for instance may be homologous to sections of mouse chromosome 6. This information may allow for genetic markers in the mouse to be used in the bovine in the search for genetic markers linked to important economic traits.

CHAPTER II

LITERATURE REVIEW

Introduction

Adipose tissue is a dynamic and essential deposit found within all living animals. The primary functions of adipose tissue are to store excess energy when there is a positive energy balance, and provide a source of high energy fatty acids in times of energy deficiency (Voet and Voet, 1990). Marbling is a deposit of adipose tissue found within the muscle and is referred to as intramuscular adipose tissue. If intramuscular adipose tissue metabolism is controlled by different mechanisms then adipose tissue metabolism in other fat deposits, then it may be possible to manipulate one adipose deposit without greatly influencing the others. This may make it possible to increase or maintain a certain level of marbling while decreasing fat in other deposit sites. Therefore, to be able to understand if marbling is under different controlling factors then other adipose tissue deposits, it is necessary to have knowledge about adipose tissue metabolism. The identification of these differences between adipose tissue sites requires detailed evaluation of enzymatic activities and hormonal stimuli that may have differing effects in different adipose tissue locations.

There is a large amount of variation seen in average marbling scores and carcass yield when we compare different breeds of cattle (Cundiff et al., 1986; reviewed by Marshall, 1993). Different breeds of cattle have diverse genetic backgrounds which is why some breeds are better adapted to certain environments. The DNA that an animal possesses is the blueprint for all of the metabolic and physiological aspects of that animal. The phenotype that an animal possesses is a combination of it's genotype and it's

environment. However, the variation observed between breeds of cattle, and within breeds, is not all explained by differing environments which suggests that a portion of the variation may be attributed to genotype. This means that there may be different genetic mechanisms controlling lipid deposition in different animals. Due to the positive correlation between overall palatability and marbling score (Smith et al., 1984; Savell et al., 1987), and the consumer's desire to have an increasingly leaner product (Breidenstein and Carpenter, 1983) it would be interesting to identify the underlying genetic reasons for the observed differences in marbling score and carcass composition seen among the cattle breeds.

The relationship amongst carcass traits is also of interest. Knowledge about how changes in one carcass trait influence other aspects of an animal's carcass composition is essential before an efficient selection program could be designed. These relationships can be evaluated through identification of genetic and phenotypic correlations. The heritability values that have been estimated for marbling and carcass composition traits would provide an indication of the efficiency of selection for marbling and carcass composition. Heritability values provide an indication of the degree of control that genetics plays on that specific trait. A high heritability value indicates that a specific trait is controlled largely by the additive genotype of an animal. The lower the heritability, the more difficult it is to make improvement through selection. Phenotypic correlations are composed of both genetic and environmental components, while genetic correlations consist of the genetic component only. Therefore, the evaluation of both phenotypic and genetic correlations can provide an indication of how important the genotype and the environment are in influencing the correlations between traits.

The use of molecular genetic techniques will aid in the identification of any such differences in the DNA sequence that may exist between and within breeds of cattle. Within carefully designed beef populations where the passage of genetic markers can be studied from parent to offspring, polymorphic regions within the DNA can be identified if

present and statistically analyzed for any significant effects on marbling and general carcass composition characteristics. Any DNA sequences which are found to have a significant effect on body composition would serve as useful tools for marker assisted selection (Lande and Thompson, 1990).

The usefulness of carcass composition as a marketing tool for the beef industry may be documented through history (reviewed by Lesser, 1993; Kempster et al., 1982). The grading of beef carcasses officially began in 1917 when the Food Production Act was passed in an effort to supply U.S. and allied troops during World War I. At this time standards for beef products were still being debated. In 1925 the "Better Beef" campaign began the practice of stamping grades on all carcasses so that consumers could discriminate between beef products. During this time frame, marbling began to emerge as a major guide to tender and juicy beef. By 1926, beef grades were stamped on carcasses on request and free of charge to all packers operating under federal inspection. In 1955 about 50% of beef carcasses were being graded. The development of dual grading (both quality and yield grading) was first established in 1960. In 1987, the 'US Good' grade was changed to 'US Select' which boosted the amount of beef voluntarily graded in this category by 700%. This increase was primarily due to consumer perceptions which resulted in a more favorable response to 'Select' graded beef compared to 'Good' graded beef. By the year 1989, the grade on a beef carcass was permitted to consist of the quality grade only, the yield grade only, or a combination of the two. Currently, the quality grades for young beef carcasses are: Prime, Choice, Select and Standard. 'US Prime', the highest quality grade, is given to meat with a minimum of slightly abundant marbling, whereas the 'US Standard' grade, the lowest quality grade, is given to meat with only trace amounts of marbling. Yield grades assigned to beef carcasses range from yield grade 1 to yield grade 5 (YG 1 - YG 5). Carcasses with minimal amounts of fat and high percentages of lean are YG 1, while carcasses with progressively higher levels of fat and lower percentages of lean are assigned progressively higher yield grades up to YG 5.

The practice of quality and yield grading beef carcasses has been utilized for approximately 77 years. Through these years, grading beef carcasses has served as a means to provide the consumer with a consistent product. The consumers can select beef products that meet their individual tastes based on expected eating quality predicted through beef grades. Breed differences exist within cattle for both quality and yield grades. These differences between breeds and within breeds (Cundiff et al., 1986) suggest there may be an underlying genetic reason for this grade variation. Through the use of molecular genetic techniques it may be possible to identify markers and/or genes associated with these grade differences. If useful markers are found, then it becomes possible to potentially use these markers in marker assisted selection.

Biology of Adipose Tissue

Adipose Tissue Metabolism

Fatty acids are an essential part of all living organisms. Fatty acids are important components of biological membranes, their derivatives serve as hormones and intracellular messengers, they provide cushioning for internal organs, and they are molecules used for energy storage (Voet and Voet, 1990). Adipose tissue deposits are dynamic regions which are constantly undergoing either fatty acid synthesis, degradation or both. The enzymes necessary for lipogenesis and lipolysis are several fold. The pathways for fatty acid biosynthesis and degradation involve different mechanisms and enzymes (Gibson et al., 1958). The process of fatty acid synthesis takes place in the cytosol of cells. The first irreversible reaction in fatty acid synthesis is performed by the enzyme acetyl-CoA carboxylase. Acetyl CoA carboxylase is responsible for the carboxylation of the acetyl-CoA molecule to produce malonyl-CoA (Wakil et al., 1983). The acetyl-CoA molecule

necessary for fatty acid synthesis is derived through other metabolic cycles. The process of glycolysis (Fothergill-Gilmore, 1986) produces pyruvate which is easily converted to produce the vast majority of acetyl-CoA used in fatty acid synthesis. The breakdown of ketone bodies and fatty acids also produce acetyl-CoA (McGarry and Foster, 1980). The enzyme system that catalyzes the synthesis of saturated long-chain fatty acids from acetyl-CoA and malonyl-CoA is called fatty acid synthase. Fatty acid synthase is actually a complex of several enzymes including acetyl transferase, malonyl transferase, condensing enzyme, β-ketoacyl reductase, dehydratase, enol reductase, and thioesterase (Bressler and Wakil, 1961; Lynen, 1961; Hsu et al., 1965). The major product of fatty acid synthase is palmitate, a sixteen carbon saturated (containing no double bonds) fatty acid. Once palmitate is formed it can be elongated and transformed into unsaturated (containing double bonds) forms to yield other fatty acids through the actions of membrane bound enzymes located on microsomes (Nugteren, 1965) and mitochondrial enzymes (Wakil, 1961, 1964). Fatty acids, once formed, are then either stored in adipose tissue deposits, or utilized as needed for normal body function.

The breakdown of fatty acids is carried out through the β-oxidation pathway within the mitochondria of the cell (Green, 1954). However, most fat is in the form of triacylglycerol and must first be broken down by lipase enzymes into glycerol and fatty acids (Desnuelle, 1968; Wills, 1965). Within adipose tissue deposits hormone-sensitive lipase (HSL) is responsible for the breakdown of triglycerides into fatty acids and glycerol (Vaughan et al., 1964; Gorin and Shafrir, 1964; Stralfors et al., 1987). The β-oxidation pathway successively oxidizes fatty acids into acetyl-CoA units which may be used in the formation of ketone bodies, or the generation of energy in the citric acid cycle. The enzymes involved in the β-oxidation pathway include an acyl CoA dehydrogenase, enoyl CoA hydratase, L-3-hydroxyacyl CoA dehydrogenase, and β-ketothiolase (Boyer, 1983).

The transport of lipid molecules within the body is facilitated through joining hydrophobic lipid molecules with hydrophilic protein molecules. The transport of lipids

from one site in the body to another is a integral part of lipid metabolism. The ingestion of lipids in the diet generally involves the breakdown of these lipids into monoglycerides and free fatty acids through the actions of pancreatic lipase and bile salts (Reiser et al., 1952). Once the monoglycerides and free fatty acids have been absorbed through the intestinal wall, they are packaged by the intestine into lipoprotein molecules called chylomicrons (Zilversmit, 1967). Lipoproteins that carry endogenous triacylglycerols and cholesterol include very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) (Atkinson and Small, 1986). These lipoprotein molecules are all broken down into their component protein and lipid parts through the action of the membrane bound enzyme lipoprotein lipase (Kompiang et al., 1976; Engelberg, 1959; Shore et al., 1959). This makes it possible for free fatty acids released from lipoprotein molecules to be taken up by adipose tissue and converted back into triacylglycerides through the process of fatty acid synthesis. Triacylglyceride is the storage form of lipids within the body. When there is a need for energy, HSL is responsible for the breakdown of triglycerides in adipose tissue into fatty acids and glycerol. When the free fatty acids are released from the adipose tissue deposits they are transferred in the blood stream through association with protein molecules. These proteins are categorized into a large group called fatty acid binding proteins (FABP). FABPs are believed to play a role as transport vehicles of hydrophobic lipid molecules throughout the cytoplasm (McCormack and Brecher, 1987; Peeters et al., 1989). Other proteins considered to be members of the FABP family include adipocyte lipid-binding protein (Baxa et al., 1989) and the myelin P2 protein (Suzuki et al., 1982). These fatty acid binding proteins help to transport the fatty acids to other tissues such as the liver, muscle, intestine, and kidney where they are oxidized through the β-oxidation pathway into usable energy.

The enzymes of lipogenesis and lipolysis, as well as the proteins necessary for the association and transport of lipid molecules in the body are all possible candidates for

having some effect on total body lipid metabolism. Polymorphisms located within the DNA coding for these enzymes and proteins could potentially effect the rates of lipolysis and lipogenesis (Ma et al., 1992; Stocks et al., 1992).

Hormonal Regulation of Lipid Metabolism

Hormonal action plays a major role in the regulation of lipid metabolism. There are several hormones which are known to influence the rate of lipid breakdown. A review of the regulation of lipid metabolism can be found in Saggerson (1980). The hormones epinephrine and norepinephrine (Gorden and Cherkes, 1958), adreno-corticotropic hormone (ACTH; White and Engel, 1958), glucagon (Hagen, 1961), and thyroid stimulating hormone (TSH; Freinkel, 1961) have all been shown to accelerate the release of free fatty acids from adipose tissue. The methods by which these enzymes function is reviewed by Voet and Voet (1990). These hormones increase the adipose tissue cyclic-AMP (cAMP) levels. The cAMP activates cAMP-dependent protein kinases which phosphorylate hormone-sensitive lipase. The phosphorylation of HSL causes an increase in this enzyme's activity which in turn increases the breakdown of triacylglycerides in adipose tissue. This increase in free fatty acids stimulates the beta-oxidation pathway which produces energy from the breakdown of FFAs. The same cAMP-dependent protein kinases which phosphorylate HSL also phosphorylate acetyl-CoA carboxylase which is the rate limiting enzyme of fatty acid synthesis. The phosphorylation of acetyl-CoA carboxylase inactivates this enzyme. Therefore, cAMP-dependent phosphorylation simultaneously stimulates fatty acid oxidation and inhibits fatty acid synthesis.

Insulin is released in response to high blood glucose concentrations. Therefore, it is not surprising that insulin helps to clear the bloodstream of extra glucose and fatty acids, through stimulating lipogenesis and inhibiting lipolysis in adipose tissue. The actions of

insulin are reviewed by Voet and Voet (1990). Insulin mediates these effects through increasing the activity of acetyl-CoA carboxylase, and stimulating the activity of lipoprotein lipase through increased synthesis of LPL. Insulin decreases the cAMP levels in adipose tissue. This leads to the dephosphorylation of hormone-sensitive lipase, which inactivates the enzyme. Insulin stimulates the activity of certain cAMP-independent protein kinases which phosphorylate proteins at different sites then cAMP-dependent protein kinases. In this manner acetyl-CoA carboxylase is activated by phosphorylation by the cAMP-independent protein kinases. The increase in LPL synthesis, the activation of acetyl-CoA carboxylase, and the inactivation of hormone-sensitive lipase all yield an increase in fatty acid synthesis and a decrease in fatty acid breakdown. The effects of hormones stimulating lipolysis are antagonistic to the effects of insulin.

Another hormone which has been shown to influence body composition is growth hormone, also referred to as somatotropin. Growth hormone when chronically maintained at physiological concentrations in beef animals increases the production of lean tissue, and decreases the total body fat content (Moseley et al., 1992; Groenewegen et al., 1990). Similar responses to growth hormone can be seen in transgenic mice with a sheep growth hormone gene (Pomp et al., 1992). This effect on an animal's total body composition is believed to occur through increased lipolysis (Davidson, 1987). Two polypeptide sequences for the growth hormone protein have been reported (Seavey et al., 1971; Wallis, 1973). Sequencing done by Zhang et al. (1992) revealed that a polymorphism within the growth hormone gene (Lucy et al., 1991) resulted in different amino acid compositions of the growth hormone protein. These results may indicate that the growth hormone polymorphism could directly be associated with body composition, or serve as a marker for it. Other compounds called β-adrenergic agonists also have been shown to alter growth rate and carcass composition of growing meat animals. The β-adrenergic agonists seem to decrease carcass fat content by decreasing lipogenesis and stimulating lipolysis (Etherton and Smith, 1991).

The effects witnessed due to hormone actions on adipose tissue metabolism would suggest that these regulating hormones are controllers of an animal's total body composition. The major players in hormonal regulation of adipose metabolism include insulin, epinephrine, growth hormone, and the β-adrenergic agonists.

Differences in Adipose Tissue Deposits

There are four primary sites of adipose tissue deposition within the animal body. These four deposits include subcutaneous adipose tissue (beneath the skin), intermuscular adipose tissue (between muscle groups), intramuscular adipose tissue (within the muscle), and perirenal adipose tissue which is found in the abdominal cavity surrounding vital organs. In order for there to be a decrease in adipose tissue located in all other deposit sites, while maintaining or increasing the adipose deposition in the intramuscular adipose deposit (marbling) there must be differences in how adipose metabolism is controlled within the different adipose tissue sites. Several studies have been done which indicate such differences exist between the location of adipose tissue sites. The cellularity of bovine adipose tissue in different deposit sites has been studied between Hereford/Angus and Holstein cattle (Hood and Allen, 1973). An increase in the size of an adipose tissue deposit is due to both hypertrophy and hyperplasia. At fourteen months of age hyperplasia appears to be complete in all but the intramuscular adipose deposits. This suggests that intramuscular adipose tissue is a late developing depot. Hyperplasia appears to be almost complete in the subcutaneous and perirenal depots of bovine animals by about 8 months of age. This data can be interpreted to mean that the rates of adipose tissue growth is related to the location of that specific adipose tissue site. Since rate of growth of adipose tissue is controlled by tissue site, the ability of different adipose tissue sites to utilize materials for growth and the way external stimuli effect that respective

deposit may also differ.

The relative contributions of acetate, lactate and glucose to lipogenesis was compared between bovine subcutaneous and intramuscular adipose tissue (Smith and Crouse, 1984). The age and diet of the animals seemed to have no effect on enzyme activities in intramuscular adipose tissue, while age and diet did have an impact on subcutaneous and perirenal adipose tissue deposits. Acetate provided 70-80% of the acetyl units to in vitro lipogenesis in subcutaneous adipose tissue, but only 10-25% in the intramuscular deposits. Glucose was found to provide 1-10% of the acetyl units in subcutaneous adipose tissue, but 50-75% in the intramuscular adipose tissue. The contribution of lactate was similar for both subcutaneous and intramuscular deposits. These data suggest that different regulatory processes control de novo fatty acid synthesis in intramuscular and subcutaneous adipose tissue. It appears that different deposit sites do respond differently to external stimuli such as age and diet, as well as preferring different building blocks for tissue growth.

Another study has compared enzyme activities between Angus (high marbling) and Santa Gertrudis (low marbling) steers. This study suggested that differences between breeds of cattle in intramuscular adipose tissue size correspond to differences in lipogenesis in vitro (Miller et al., 1991). Results shown indicate that enzymes necessary to produce NADPH and glycerol (pentose cycle reductase and NADP-malate dehydrogenase) for fatty acid biosynthesis and triacylglycerol esterification had higher activities in Angus intramuscular adipose then in Santa Gertrudis intramuscular adipose tissue. Therefore, the enzymes necessary to produce molecules for lipolysis seem to work at different rates in different breeds of cattle. This could suggest one reason why different breeds of cattle tend to marble at different levels.

The effect of feeding the β-adrenergic agonist clenbuterol to heifers also yielded some interesting results on lipid deposits (Miller et al., 1988). Basal lipolysis in intramuscular adipose was approximately 55% of the rate observed in subcutaneous

adipose tissue, and appeared to be unaffected by clenbuterol treatment. Clenbuterol treatment depressed lipogenic enzyme's and fatty acid binding protein's activities as well as decreasing the rate of basal lipolysis in the subcutaneous depot. These findings point to differences between the effects of the β-adrenergic agonist clenbuterol on adipose metabolism in the different adipose tissue sites, even though treatment with clenbuterol in general caused a decrease in both U.S.D.A. quality and yield grades in treated animals. The fact that differences in effect of the beta-adrenergic agonist were witnessed between different adipose deposits provides further evidence that lipid metabolism is under varied control depending on location of the adipose deposit.

The results discussed here indicate that adipose tissue in general does not all respond in the same manner. It would appear that the deposit site of the adipose tissue has some influence on its metabolic rate and its response to external compounds such as clenbuterol. Evidence such as this would support the idea that different adipose tissue sites may be manipulated differently. This means that marbling scores could be maintained while decreasing subcutaneous, perirenal, and intermuscular adipose deposits. Multi-trait selection based on different adipose tissue sites could potentially yield a beef market animal with a predetermined minimum quality grade to produce desirable palatability attributes and a low yield grade.

Genetic Relationships

Economic Importance of Marbling

The correlations between marbling scores and several palatability attributes such as flavor, juiciness and tenderness have long been debated. Most research has found that marbling score and tenderness are lowly correlated. Marbling score has negative

phenotypic correlations with Warner-Bratzler shear force with values of -.12 and -.18 (Koch, 1978; Van Vleck et al., 1992) and has a positive phenotypic correlation with sensory panel tenderness values of .19 (Van Vleck et al., 1992). These values support the idea that marbling is associated with a more tender meat product, however, this association appears to be very weak. Four reviews have been made of the then-existing research data involving relationships between marbling and beef palatability. In 1963 Blumer concluded that marbling explained about 5% of the variability in tenderness and about 16% of the variation in juiciness. Conclusions were made by Jeremiah et al. (1970) that marbling was associated with 2 to 16% of the variability in flavor, juiciness, tenderness and overall palatability. Another review in 1974 by Smith and Carpenter concluded that marbling had low to moderate relationships with flavor, juiciness and tenderness of beef. In 1984 a comprehensive study was performed in collaboration with the USDA and three land grant Universities to determine the relationships of USDA quality grades to palatability of cooked beef (Smith et al., 1984). It was found that steaks from carcasses with higher marbling scores were more desirable in mean flavor ratings in 31.7% of comparisons with steaks from carcasses with lower marbling scores. The same comparison between low and high marbling scores yielded percentages of 39.3% for juiciness, 18.8% for amount of connective tissue, 35.7% for tenderness, 35.3% for overall palatability and 27.7% for shear force value. About one-third of the time carcasses with higher marbling scores produced loin and round steaks that were more flavorful, juicy, tender and palatable than those from a carcass with a lower marbling score. Results shown in these studies and reviews of the literature on marbling and palatability reflect the fact that marbling does have an impact on sensory attributes that compose overall palatability of meat products, however this impact appears to be fairly small. The fact remains that regardless of the relatively small effect that marbling score has on predicting a piece of meat's eating qualities, it is the best visual indicator that is presently available and easy to use. For these reasons marbling score will probably continue to be an indicator of

meat quality until a better evaluation method can be found.

Heritabilities and Correlations

The average heritability values for carcass characteristics and the correlations between these carcass traits can provide useful information by indicating how successful selection will be, and providing an indication of how an animal's genotype and environment affect these traits. Table 1 presents several heritability values for a number of carcass traits. The range of standard errors associated with the heritability values in Table 1 are .04 to .25. The highest standard errors are associated with the heritabilies calculated by Koch (1978). Table 2 presents the correlations between the carcass traits. The range of standard errors associated with the genetic correlations found in Table 2 are from .04 to .99. The extreme standard error of $\pm$ .99 is associated with the genetic correlation between marbling score and rib-eye area calculated by Koch (1978). In general it appears that most carcass traits of interest have moderate heritabilities. Such heritabilities suggest that it would be possible to make respective gains using selection to improve these traits. However, there are unfavorable correlations between some of the carcass traits which we would like to select for. Increasing carcass weight tends to have the unfavorable relationship of increasing fat depth. Rib-eye area has an average negative genetic correlation with marbling score and with sensory panel tenderness. However, the phenotypic correlations between rib-eye area and these same traits are much more neutral. Marbling score is positively correlated with fat depth. These unfavorable relationships make selection for any one carcass trait without consideration for the others a disadvantage. Evidence has also been shown that the phenotypic correlations between carcass traits such as carcass weight, rib-eye area, fat depth, and marbling score are all positively correlated with the production traits of weaning weight and post-weaning gain

(Koch et al., 1982; Lamb et al., 1990). This would mean that selection for increased growth would mean beneficial increases in most carcass traits except for the increase in fat depth.

Table 1. Heritabilites for Several Carcass Traits

HCW	REA	FAT	MARB	W.B. SH.	SEN. TEN.
.68(1)	.28(1)	.68(1)	.34(1)	.31(2)	.10(9)
.43(2)	.56(2)	.41(2)	.40(2)	.71(8)	
.48(3)	.40(3)	.52(3)	.47(3)	.09(9)	
.44(4)	.28(5)	.24(5)	.33(5)		
.31(5)	.01(7)	.52(6)	.45(9)		
.33(7)	.60(9)		.23(10)		

HCW = Hot carcass weight; REA = Rib-eye area; FAT = Subcutaneous fat thickness; MARB = Marbling score; W. B. SH. = Warner-Bratzler shear force value; SEN. TEN. = Sensory tenderness values. Numbers in parentheses represent the respective reference for the presented heritability value. 1- Koch, 1978; 2- Koch et al., 1982; 3- Benyshek, 1981; 4- MacNeil et al., 1984; 5- Lamb et al., 1990; 6- MacNeil et al., 1991; 7- Reynolds et al., 1991; 8- Shackelford et al., 1992; 9- Van Vleck et al., 1992; 10- Woodward et al., 1992.

These correlations and heritability values are all useful indicators which tell us how the genotype and environment of an animal affects carcass composition. The carcass traits presented here all have moderate heritability values which suggests that selection for these specific traits could be successful. There appears to be obvious differences seen between phenotypic and genotypic correlations which tells us that the environment plays a part in influencing body composition. However, the level of genetic impact on carcass traits is evidenced in the heritability values and the genetic correlations. This relationship between genotype and body composition suggests that there are underlying genetic reasons to explain the breed differences observed in beef cattle. Therefore, DNA tests which identify polymorphisms within cattle breeds could be potentially useful selection tools for carcass

composition in beef cattle.

Table 2. Genetic (below diagonal) and Phenotypic (above diagonal) Correlations Among Carcass Traits

TRAITS	HCW	REA	FAT	MARB	W. B. SH.	SEN. TEN.
HCW		.37(1)	.42(1)	.18(1)	.00(2)	
		.43(2)	.36(2)	.13(2)		
		.58(5)	.38(5)	.28(5)		
REA	.02(1)		-.08(1)	-.03(1)	-.02(2)	.00(9)
	.44(2)		-.15(2)	.03(2)	-.05(9)	
	.68(5)		.04(5)	.19(5)		
				.00(9)		
FAT	.95(1)	.03(1)		.25(1)	-.01(2)	
	.08(2)	-.44(2)		.24(2)		
	.14(5)	-.04(5)		.38(5)		
MARB	-.33(1)	-1.34(1)	.73(1)		-.12(1)	.19(9)
	.25(2)	-.14(2)	.16(2)		-.18(9)	
	.64(5)	.57(5)	.73(5)			
		-.40(9)				
W. B. SH.	.00(2)	-.28(2)	.26(2)	-.25(2)		-.70(9)
		-.14(9)		-.53(9)		
SEN. TEN.		-.04(9)		.74(9)	-.96(9)	

HCW = Hot carcass weight; REA = Rib-eye area; FAT = Subcutaneous fat thickness; MARB = Marbling score; W. B. SH. = Warner-Bratzler shear force value; SEN. TEN. = Sensory tenderness values. Numbers in parentheses represent the respective reference for the presented correlation value. 1- Koch, 1978; 2- Koch et al., 1982; 5- Lamb et al., 1990; 9- Van Vleck et al., 1992.

Variation Within and Between Breeds

It has been known for many years that different breeds of beef cattle are superior for different types of production goals. The variation within a breed of cattle is also witnessed by the fact that within one breed of cattle many possible expected progeny difference (EPD) values can be found as in any breed's sire summary. This variation in all cattle populations, purebred or crossbred, makes selection possible. Therefore, variation among cattle is necessary for carcass traits before selection can be used to improve body composition of beef animals. Evidence supporting the fact that variation exists between different breeds of cattle and within breeds of cattle is presented in Cundiff et al. (1986). Variation was reported for carcass weight, fat thickness, kidney fat, marbling score, and retail product produced. Sire summaries also provide evidence for additive genetic variation within a breed of cattle. These sire summaries present the EPDs for several economically important traits including both production traits and sometimes carcass traits. The Angus sire summary is the only purebred beef cattle breed that publishes carcass EPDs. The carcass EPDs represented within the Angus sire summary express the variation within a breed of cattle for a carcass trait such as marbling, rib-eye area, hot carcass weight and fat thickness.

The desire to maintain a certain level of marbling and decrease all other fat deposits within our market animals creates a dilemma for the beef producer. In order for the beef industry to select for this market animal phenotype we must first establish the fact that all fat deposits do not respond the same. Other requirements before selection can be successful are that variation exists both within and between the breeds of cattle today. Since the literature supports that fact that heritability values are in the moderate range, and that variability between and within breeds does exist for carcass traits we have evidence to suggest that selection for desirable body composition is possible (Mills, 1994; Shoup,

1994).

Molecular Genetics of Adipose Metabolism

The quantity of molecular genetic information available for bovine is rapidly growing; however, DNA sequence and genetic marker information is limited at this point in time. Any DNA sequence information available for the enzymes and hormones that are involved in adipose metabolism control are valuable links to understanding why different animals have differences in their adipose tissue deposits. This chapter will briefly review the molecular information available for proteins of interest which have known effects on lipid metabolism in other species as well as in the bovine.

Molecular genetic information for LPL has been reviewed in a number of different species. The enzyme LPL is a member of a highly homologous gene family of serine esterases including hepatic lipase and pancreatic lipase (Kirchgessner et al., 1989). The major function of LPL is to hydrolyze the triglyceride core of circulating chylomicrons and very low density lipoproteins, which results in the release of free fatty acids (Brunzell, 1989). The human LPL gene is composed of ten exons spanning approximately 30 kilobases (Kirchgessner et al., 1989). The first exon encodes the 5'-untranslated region, the signal peptide plus the first two amino acids of the mature protein (Kirchgessner et al., 1989). The next eight exons encode the remaining 446 amino acids, and the tenth exon encodes the long 3'-untranslated region of 1,948 nucleotides (Kirchgessner et al., 1989). The three-dimensional structure of human pancreatic lipase has been studied and an Asp-His-Ser triad has been proposed as the catalytic site (Winkler et al., 1990). The human LPL gene also contains such an Asp-His-Ser triad. Recent results of *in vitro* site-directed mutagenesis studies on eight completely conserved serine residues, including the Ser^{132}

residue believed to be the Ser residue in the catalytic triad, support the central role of Ser^{132} in LPL catalysis (Faustinella et al., 1991). In addition, two missense mutations at the first and second nucleotides of the codon for Asp^{156} (also in the catalytic triad) in the human LPL gene have also been found to cause a catalytically defective LPL (Ma et al., 1992). Research has also indicated that certain exon regions of the LPL gene may be especially sensitive to mutations. Studies have been done on human patients with LPL deficiency, and six of the eight missense mutations that have been reported to cause a catalytically defective LPL protein are located at completely conserved residues in exons 4, 5, and 6 of the human LPL gene, providing in vivo evidence that these residues are important for LPL catalysis (Ameis et al., 1991; Beg et al., 1990; Monsalve et al., 1990; Emi et al., 1990; Henderson et al., 1991; Dichek et al., 1991; Hata et al., 1990). Several additional polymorphisms have been found in the human LPL gene. Three separate polymorphisms in introns 3, 6, and 8 of the human LPL gene were detected (Gotoda et al., 1992). A premature termination codon mutation was detected in human LPL in exon nine which appeared to have no phenotypic effect (Stocks et al., 1992). Other mutations in the human LPL gene have been associated with familial chylomicronemia (Reina et al., 1992) and cardiovascular disease (Ahn et al., 1993). The structure and polymorphic map of the human LPL gene have been presented by Oka et al. (1990). The cDNA sequence of LPL has been published for several species, including the mouse (Kirchgessner et al., 1987), rat (Brault et al., 1992), guinea-pig (Enerback et al., 1987), chicken (Cooper et al., 1989), porcine (Harbitz et al., 1992) and bovine (Senda et al., 1987). The porcine LPL cDNA sequence has been used as a probe to detect three restriction fragment length polymorphisms in porcine genomic DNA (Harbitz et al., 1992).

The coding region of LPL is strongly conserved among species, and a high degree of homology between LPL, hepatic lipase and pancreatic lipase suggests the existence of a lipase gene family (Komaromy and Schotz, 1987). This information along with the evidence of polymorphisms found within the human and porcine LPL gene sequence

suggest that there would be a reasonable chance of polymorphisms existing in the bovine LPL gene. The fact that cDNA sequence information is available for the LPL gene in bovine, and that the human and bovine LPL genes are highly homologous makes the bovine LPL gene a possible candidate as a genetic marker for carcass traits in cattle.

LPL is responsible for the breakdown of triglycerides found in the bloodstream into free fatty acids which can be taken up by the adipose tissue and stored (Kompiang et al., 1976; Engelberg, 1959; Shore et al., 1959). When there is a need for these free fatty acids as an energy source the adipose tissue releases them through the action of hormone sensitive lipase (Vaughan et al., 1964; Stralfors et al., 1987). These free fatty acids are then transported through the bloodstream in complexes with fatty acid binding proteins (Peeters et al., 1989). Sequence information is available in several species including bovine for fatty acid binding protein (Billich et al., 1988). This makes FABP another gene of interest as a possible genetic marker for carcass traits in beef animals.

Smith et al. (1988) first reported a positive correlation between longissimus muscle FABP activity and the marbling score of the bovine longissimus dorsi muscle. Later reports have indicated that this earlier correlation was caused due to adipose tissue contamination of samples, and that the positive correlation between marbling score and muscle FABP activity is not valid (Moore et al., 1991). However, a detailed study of this relationship has not definitively confirmed the association between FABP activity and marbling score. The cDNA sequence which comprises the entire information of the amino acid sequence of heart-FABP (H-FABP) is available for bovine (Billich et al., 1988). Sequence data for human heart and skeletal FABP have indicated that these two are identical proteins and exist in only one form (Peeters et al., 1991). Also of interest is the fact that bovine H-FABP has a high degree of amino acid sequence similarity to human muscle FABP (Peeters et al., 1991). These results suggest that there is a possibility that the bovine H-FABP gene may be identical to the bovine skeletal muscle FABP gene. The bovine skeletal muscle FABP gene has not yet been sequenced.

The cDNA of bovine H-FABP includes 236 nucleotides of the 3'-nontranslated region and contains the polyadenylation signal which proceeds the poly(A) tail by 24 nucleotides (Billich et al., 1988). The bovine cDNA also includes the eukaryotic translation initiation consensus sequence found in the 5' end, tryptic peptides 4, 8, 10 and 15, as well as peptide 16 (Billich et al., 1988).

The effects that growth hormone has on body composition and body metabolism of protein and lipids (Groenewegen et al., 1990; Press, 1988) make this gene and its regulatory regions of interest. The chromosomal gene for bovine growth hormone has been sequenced (Gorden et al., 1983). Two polypeptide sequences for the bovine growth hormone gene have been reported (Seavey et al., 1971; Wallis 1973). Lucy et al. (1991) first reported a polymorphism within the bovine growth hormone gene which was detected by polymerase chain reaction (PCR) and endonuclease digestion. This polymorphism revealed two alleles for the bovine growth hormone gene. The question was then raised as to whether or not this DNA polymorphism was actually the underlying reason for the heterogeneity of the bovine somatotropin polypeptide sequence. Sequencing of the B allele done by Zhang et al.(1992) which was based on the polymorphism first reported by Lucy et al. (1991) revealed that a cytosine was substituted by a guanine at the fourth base of exon 5. This resulted in the production of the B peptide in which a valine was substituted for a leucine at amino acid position 127 (Seavey et al., 1971). Therefore, a polymorphism exists in the coding region of the bovine growth hormone gene which results in the change of one amino acid in the growth hormone protein. This growth hormone polymorphism could have direct effects on an animal's body composition due to the fact that two different forms of the growth hormone protein are produced depending on the alleles for growth hormone that an animal possesses. A growth hormone-*Taq I* polymorphism was identified in cattle by Theilmann et al. (1989). Another allelic fragment for the growth hormone-*Taq I* marker in cattle was found by Rocha et al. (1992a). This polymorphism was identified through RFLP analysis of a cDNA clone encoding bovine

growth hormone (Rocha et al. 1992a). The use of single stranded conformational polymorphisms (SSCP) identified three alleles in the fourth intron of the bovine growth hormone gene (Kirkpatrick, 1992). These polymorphisms all hold the possibility of being associated either directly with or as genetic markers for carcass characteristics in cattle.

The transcription of the growth hormone gene is regulated by several transcription factors in humans and rats (Ingraham et al., 1990; Karin et al., 1990). Growth hormone is produced primarily by somatotroph cells in the anterior pituitary (Wantanabe and Daikoku, 1979). The pituitary-specific expression of growth hormone is driven by the interaction of the transcription factor Pit-1 (also referred to as GHF-1) with two binding sites in the proximal promoter of the growth hormone gene (Ingraham et al., 1990; Karin et al., 1990). Pit-1 is characterized as a POU-domain-specific protein (reviewed by Rosenfeld, 1991). In general, the POU-domain transcription factors exert critical functions in the proliferation of specific cell types, as well as in the activation of specific programs of gene expression that define specific cell phenotypes within an organ (Rosenfeld, 1991). These descriptions of the Pit-1 protein are useful in explaining the effect Pit-1 has on the expression of growth hormone. The deletion of growth hormone expression in somatic cell hybrids is tightly linked to the disappearance of Pit-1 (McCormick et al., 1988). However, the addition of purified Pit-1 to extracts of GH-nonexpressing cells leads to activation of the growth hormone promoter (Bodner and Karin, 1987). When these results are considered together, they implicate Pit-1 as the major determinant of cell type-specific expression of the growth hormone gene (Bodner et al., 1988). Therefore, since the growth hormone gene is of interest to bovine body composition, it stands to reason that the Pit-1 gene should be as well. Without the expression and proper binding of the Pit-1 transcription factor to the promoter region of the growth hormone gene, there would be no expression of growth hormone (McCormick et al., 1988).

Sequence information for Pit-1 is available in human, rat, bovine and swine. The

human Pit-1 gene was sequenced by Ohta et al. (1992), the rat and bovine cDNA sequence was determined by Bodner et al. (1988) and a partial swine cDNA was sequenced by Tuggle et al. (1993). Polymorphisms within the Pit-1 gene have been identified in swine. The Pit-1 gene has polymorphic *Bam* HI fragments in pure-bred Meishan swine, but only monomorphic fragments in five American swine breeds (Tuggle et al., 1993). A *Msp*I restriction fragment length polymorphism at the swine Pit-1 locus was also identified (Yu et al., 1993). The availability of sequence information for bovine cDNA and the evidence that polymorphisms have been found in the highly conserved POU-domain protein (Herr et al., 1988) Pit-1, combined with the obvious role that Pit-1 has in influencing growth hormone transcription provide support for evaluation of the Pit-1 gene as a possible marker for beef carcass traits (Moody and Pomp, 1994).

Although the four genes briefly mentioned in this chapter are far from all of the possible genes that could have controlling effects on a beef animal's body composition, they provide a good start in the search for genetic markers linked to bovine body composition. All four genes, LPL, H-FABP, GH and Pit-1, have cDNA sequence information available for bovine. This information, combined with sequence data known in other related species to the bovine, makes it possible to attempt PCR and then search for polymorphisms in the DNA that may be detected through molecular genetic techniques.

Marker Assisted Selection

Quantitative traits are those which have continuous variation such as body weight, average daily gain and fat thickness (Falconer, 1965). Qualitative traits are those with threshold characters such as horned or polled (Falconer, 1965). Quantitative traits in animal production are believed to be inherited based on the polygenic model. This model

assumes that the breeding value an animal has for a particular quantitative trait is the sum of small and additive effects of many genes (Hoeschele, 1988). In current animal breeding systems the prediction of genetic differences between animals is based on phenotypic observations which are composed of environmental and genetic components (Meuwissen and Van Arendonk, 1992). The use of molecular genetic techniques such as restriction fragment length polymorphisms (RFLP; Botstein et al., 1980) and the polymerase chain reaction (PCR; Saiki et al., 1988) now make it possible to identify genetic differences at the DNA level. These differences, which are called genetic markers, are not likely to be quantitative trait loci (QTL) themselves, but they may be linked to QTL (Soller, 1978). When marker data are integrated with traditional methods for selection, the accuracy of selection may be increased as well as allowing selection to be made at very young ages which yields a substantial increase in efficiency (Lande and Thompson, 1990). Selection which is based on both traditional methods, as well as the use of genetic markers is referred to as marker-assisted selection (MAS).

The best approach for mapping QTL through the use of genetic markers is widely debated, however, many general concepts have been established. The idea of following the transmission of chromosomes from parent to offspring through the use of gene markers was first proposed by Thoday (1961). Recent studies have evaluated the type of experimental design best suited to follow the passage of gene markers from parent to offspring, and to make associations between gene markers and QTL. Determining marker-QTL linkage requires examining offspring of individuals heterozygous at both the marker locus and the QTL (Soller, 1990). Theoretical studies show that experiments based on crosses will be most effective for mapping quantitative trait loci affecting traits whose value differs markedly between populations (Soller, 1990). Experiments based on analyses of progeny groups within sires will be most effective for mapping quantitative trait loci affecting traits showing considerable within-population genetic variation (Soller, 1990).

Several statistical methods have been proposed in order to identify QTL through the use of genetic markers. Geldermann (1975) described a model which allowed for the inclusion of several marker loci at the same time and was designed to be used for animals with only small groups of offspring per parent. Geldermann (1975) assumed that a marker allele represents a section of a chromosome when its transmission from parent to progeny is noted. Therefore, an allele substitution of the marker gene also denotes a substitution of the chromosome section concerned. It is expected that few of the marker genes themselves influence the quantitative trait considered. However, the marker gene can be linked to one or several loci, the alleles of which influence the development of a distinct quantitative trait. Geldermann's model is able to provide relations between single chromosome sections and a quantitative character. This procedure permits a continuously varying character to be traced back to a series of effects connected with marked chromosome sections.

Dairy cattle breeding has mainly utilized daughter and granddaughter designs to link genetic markers with QTLs (reviewed by Weller et al., 1990). The progeny of a sire heterozygous for both a marker locus and a linked QTL, which inherit different alleles for the marker, will have different trait means. The daughter design involves the study of marker genotype and quantitative trait values which are assessed on daughters of sires heterozygous for the markers. This design would work as well for any progeny, male or female, as long as both the marker genotype and the quantitative trait of interest were measurable in the sire's progeny. The granddaughter design involves attaining the marker genotype in sons of heterozygous sires and measuring the quantitative trait (i.e. milk production) in daughters of these sons. The use of genetic linkage maps consisting of codominant DNA markers, which are typically restriction fragment length polymorphisms, is another widely used method of mapping genetic markers to QTLs (Lander and Botstein, 1989). Lander and Botstein (1989) were the first to propose the mapping of QTL through the method of interval mapping which is widely applied and successfully used in a number

of specific case studies (e.g., Paterson et al., 1988, 1991; Stuber et al., 1992). Therefore, the use of RFLP derived genetic linkage maps serves as a useful tool for mapping QTL. The use of information from flanking markers to estimate the position and size of the effect of a QTL lying between two markers is another useful method to identify QTLs (Martinez and Curnow, 1992). Lande and Thompson (1990) proposed a method of MAS which, rather than actually mapping QTLs, used multiple regression of the phenotype on markers to identify a set of markers associated with QTLs as well as to estimate the marker effects. Another approach, using the animal model for BLUP (Fernando and Grossman, 1989; Goddard, 1992), estimates an effect associated with each marker allele in each individual. In summary, there are several methods employed to map QTL through the use of genetic markers. Each has its own set of advantages and disadvantages. With the long list of publications, some listed above, it seems that the mapping of QTL through the use of genetic markers is a reality. Therefore, we must now ask the question, of what use can marker-assisted selection be for the livestock industry?

The usefulness of marker-assisted selection has been discussed many times within the published literature. The following paragraph will review the potential benefits of MAS. The detection of marker loci linked to major genes or QTL of large effect in farm animals is of great potential value, both because it allows the easy manipulation of the major genes and because it provides a possible route to their ultimate isolation (Haley, 1991). The potential efficiency of marker assisted selection on a single trait utilizing a combination of molecular and phenotypic information depends on the heritability of the trait, the proportion of the additive genetic variance associated with the marker loci and the selection scheme (Lande and Thompson, 1990). The relative efficiency of MAS is greatest for characters with low heritability, if a moderate or large fraction of the additive genetic variance is significantly associated with the marker loci (Lande and Thompson, 1990). The use of MAS selection is also advantageous over traditional selection when markers can be found that identify traits only expressed in one sex (Lande and Thompson,

1990). The selection of markers associated with maternal ability could greatly decrease the time and money needed to estimate a sire's breeding value through traditional progeny testing. Typical animal breeding involves the estimation of an animal's breeding value through the use of individual and family records. MAS may have a higher relative efficiency than traditional animal breeding selection practices if there are common family environmental effects (Lande and Thompson, 1990). The use of MAS would minimize the bias that exists when measuring phenotypic traits which can be more drastically affected by the environment then the genetic components of an animal. The use of MAS could increase the accuracy of selection, and marker data can be collected early in life which allows selection at earlier ages (Meuwissen and Van Arendonk, 1992).

Beckmann and Soller (1983) did a study to see if identification and mapping of QTL and marker-assisted early selection of animals in livestock improvement programs was cost effective. They found that in most cases, the anticipated costs appear to be commensurate with the scientific or economic value of the application (Beckmann and Soller, 1983). Gimelfarb and Lande (1994) performed a computer simulation based on an index that incorporates both phenotypic and molecular information. The molecular markers contributing to the index and their relative weights are determined by multiple regression. It was demonstrated that selection based on genetic markers can effectively utilize the linkage disequilibrium between genetic markers and QTLs created by crossing inbred lines (Gimelfarb and Lande, 1994). Selection was more efficient if markers contributing to the index are re-evaluated each generation.

Some comparisons between MAS and more traditional methods of animal selection do not provide positive outlooks for the practical use of MAS. Meuwissen and van Arendonk (1992) assumed that marker effects are due to clusters of QTL, each QTL having a small effect. They interpreted this to mean that gene frequency changes of individual QTL will be small, and, thus, genetic variance will not decrease much because of changes in gene frequencies. Therefore, in the long term, both conventional and MAS

selection methods would fix the positive allele of the QTL and would achieve the maximum possible response for a given QTL (Meuwissen and van Arendonk, 1992). This means that MAS would mainly increase the gene frequency of QTL with large effect, and it would only be superior over conventional selection in the short term, less than $\pm$ 5 generations (Saefuddin and Gibson, 1991). Computer simulation of MAS using linkage disequilibrium was attempted by Zhang and Smith (1992). Computer simulation of selection was done with MAS, conventional BLUP methods and a combination of both MAS and conventional BLUP methods. Results showed that genetic gains by conventional BLUP selection were usually greater than by MAS. However, Zhang and Smith (1992) suggested that detection of markers closer to the QTL sites would increase the linkage disequilibrium available for selection. Eventually with very close linkage to each QTL, MAS selection would be equivalent to selection on the QTLs themselves (Zhang and Smith, 1992).

There are a number of genetic markers that have already been associated with QTL in livestock production. The halothane gene is a good example of how MAS is presently being used in the livestock industry. Porcine stress syndrome (PSS) and pale soft exudative (PSE) pork both represent significant losses to the swine industry (Hubbard et al., 1990). Both of these conditions have been genetically related to the reaction to halothane anesthesia (Webb et al., 1982). The identification of a mutation in the ryanodine receptor which has a perfect correlation to animals expressing malignant hyperthermia (the disease responsible for PSE and PSS) made marker assisted selection a reality in the swine industry (Houde et al., 1993). Today, some large hybrid swine breeding companies have integrated molecular testing for the halothane gene into there selection practices.

Two milk protein genes, kappa-casein (Medrano and Aguilar-Cordova, 1990a) and beta-lactoglobulin (Medrano and Aguilar-Cordova, 1990b) have polymorphisms within the protein coding regions of the genes. This results in two possible forms of the protein being produced depending on which alleles of the gene a respective animal has. The

casein family of milk proteins and beta-lactoglobulin significantly influence the composition and physical-chemical properties of milk, and have been associated with the quality and quantity of cheese derived from milk (Schaar et al., 1985). Cowan et al. (1992) estimated chromosome substitution effects for allelic variants of kappa-casein and beta-lactoglobulin on transmitting abilities for yield traits among sons of two Holstein sires. They concluded that allelic variants of kappa-casein and beta-lactoglobulin can be used to detect chromosome substitution effects for yield traits within sire families of Holstein cattle.

In sheep, the Booroola fecundity gene (Fec^B) influences ovulation rate and litter size (reviewed by Bindon, 1984). Homozygotes (BB), heterozygotes (B+) and non-carriers (++) of the Fec^B gene are segregated on the basis of ovulation rate recordings of $\geq$ 5, 3 or 4, and 1 or 2, respectively (Davis et al., 1982). These findings suggest that the use of the Booroola Fec^B gene in a MAS program could yield large benefits to the sheep industry in terms of increasing the prolificy of some domestic breeds of sheep.

Other studies have found associations between gene markers and economically important livestock traits. Three growth hormone-*Taq I* alleles were associated with decreases in birth weight, as a maternal trait, and decreases in shoulder width at birth (Rocha et al., 1992b). Within this same study, a significant association between the parathyroid hormone-*Msp I* marker and measures of body size were detected, as well as an effect on weaning weight as a maternal trait. Other QTL studies have identified significant associations between genetic markers located on swine chromosome 4 with growth rate and fatness (Andersson et al., 1994). Results such as these indicate that the identification of QTL regions in livestock species is possible, and is currently being done through the use of genetic markers.

The increase in markers being identified have resulted in the growth of the bovine genome maps (Barendse et al., 1994, Bishop et al., 1994). With the increase of markers with known locations on bovine chromosomes, there becomes a much larger chance of

identifying markers significantly associated with economically important traits. The recent surge in the search for markers, and the continued progress of the genome mapping groups should increase the feasibility of using marker assisted selection.

In general, the use of genetic markers is expected to accelerate genetic progress through increasing accuracy of selection, reduction of generation interval and increasing selection differentials (Soller and Beckmann, 1983; Kashi et al., 1990; Meuwissen and van Arendonk, 1992). At this time, the most useful genetic markers are those that have a large effect on specific traits of economic importance. The number of genetic markers with this type of effect are few. The practical use of MAS in commercial livestock situations is still unfounded; however, substantial increases in the efficiency of artificial selection through improved technology will increase the feasibility of MAS (Lande and Thompson, 1990). Before MAS can become a reality there will need to be more extensive testing of the new markers that are being found at increasingly faster rates. With continued research and an increase in the amount of specific associations between genetic markers and economically important traits, there is great potential for the use of MAS.

Summary

The beef cattle industry is challenged to produce a meat product that meets the consumer's demands for a lean, healthy, flavorful and tender product. To meet all of these demands is a difficult task, due to the fact that there are negative correlations between lean and palatable meat products. The literature presented here suggests that there are indeed different controlling factors for lipid metabolism between different adipose tissue sites. This provides support for the idea that most fat deposits can be decreased, while the marbling score of an animal can be increased or maintained to insure a certain level of eating quality. The use of MAS to accomplish the goal of producing a lean yet palatable

meat product is possible if genetic markers which have significant and differential impacts on carcass traits can be identified.

The literature also provides sequence information for certain genes of interest that may have controlling roles in lipid metabolism. This information makes the amplification of these gene sequences and the search for genetic polymorphisms a real possibility through the use of PCR and other molecular genetic techniques. If genetic markers are identified that have significant influences on carcass traits it may then be possible to experiment with the potential benefits gained to the beef industry through the use of MAS to produce the ideal market animal.

CHAPTER III

MATERIALS AND METHODS

Population Design

The original development and design for the beef cattle resource population used in this study was done by animal scientists at the University of Nebraska-Lincoln. Production (Appendix A), meat science (Appendix B) and reproductive physiology studies (Appendix C) were all done using this population. The appropriate reference literature and results for these studies can be found in their respective appendix sections.

Twelve bulls were selected from the 1989 Angus sire summary with accuracies greater than or equal to .50 for marbling score EPD. Six sires had high EPDs (1989 mean = +.62), and six sires had low EPDs (1989 mean = -.23) for marbling score. Table 3 and Table 4 provide names, registration numbers, current EPD values and accuracies for all twelve Angus sires. In addition to the original twelve sires, two other Angus sires for each the high and low marbling groups were selected to provide additional EPD information (Table 3 and Table 4, respectively). Explanation of how the American Angus Association qualifies marbling is found in Appendix D.

The original 12 sires were mated at random to composite MARC II cows (1/4 Angus, 1/4 Hereford, 1/4 Gelbvieh and 1/4 Simmental) to produce offspring in a two year period (1990 & 1991). Steers (n=120) and heifers (n=120) were weaned and fed to slaughter weights. Heifers were placed on a ration to gain 1.1 to 1.4 lbs/day while they were utilized for the reproductive study discussed in Appendix C. Due to involvement in the reproductive study, heifers were at heavier starting weights when they were placed on the feedlot trial rations. Steers were placed directly on feedlot trial rations after weaning.

Table 3. Current EPD values for Bulls in the High Marbling EPD Group.

Name & Registration #	BW	DWW	YW	HCW	MARB	REA	FAT
Premier Independence K N	+4.0	+16	+22	+4	+.28	+.27	-.02
#09958208	(.97)	(.97)	(.96)	(.91)	(.90)	(.90)	(.88)
S A Direct Drive 83	+6.7	+23	+35	+11	+.39	+.07	-.04
#10383408	(.90)	(.90)	(.85)	(.73)	(.70)	(.70)	(.67)
R&J Justice 1359	+4.3	+31	+51	-6.0	+.24	+.10	-.04
#09591236	(.86)	(.85)	(.81)	(.55)	(.51)	(.51)	(.49)
Paramount Ambush 2172	+0.7	+30	+50	+1.0	+.14	+.15	-.03
#10239760	(.91)	(.90)	(.85)	(.72)	(.69)	(.69)	(.66)
VDAR Receiver	+3.9	+23	+42	-6.0	+.12	+.01	-.02
# 10281397	(.78)	(.75)	(.67)	(.58)	(.55)	(.55)	(.52)
P S Sasquatch 904	+4.3	+31	+54	+8.0	+.06	+.24	-.01
#09459638	(.97)	(.96)	(.95)	(.88)	(.87)	(.87)	(.86)
*H M H Commodore 100	+0.7	+9.0	+7.0	-24	+.28	-.13	-.03
#06855486	(.84)	(.88)	(.81)	(.82)	(.79)	(.79)	(.76)
*Band 234 of Ideal 3163	+0.8	+12	+32	+3.0	+.26	+.10	-.01
#08505294	(.96)	(.96)	(.95)	(.88)	(.87)	(.87)	(.87)

*Bulls that were selected by Oklahoma State University researchers to supplement EPD data. All EPD values are provided by the National Angus Association 1994 Sire Evaluation. Accuracy values are located in ( ) under EPD values. BW=birth weight EPD, DWW=direct weaning weight EPD, YW=yearling weight EPD, HCW=carcass weight EPD, MARB=marbling EPD, REA=rib eye area EPD and FAT=fat depth EPD.

Table 4. Current EPD Values for Bulls in the Low Marbling EPD Group.

Name & Registration #	BW	DWW	YW	HCW	MARB	REA	FAT
Nichols Landmark L56	+6.1	+35	+65	+21	-.40	-.08	+.04
#09586830	(.97)	(.96)	(.95)	(.90)	(.88)	(.88)	(.87)
Prairielane DoubleImage5002	+6.2	+47	+70	+9.0	-.20	+.35	-.01
#10769980	(.84)	(.83)	(.79)	(.66)	(.64)	(.64)	(.61)
Jetliner 707 of Conanga	+3.8	+24	+45	+14	-.21	+.38	-.02
#07795918	(.96)	(.96)	(.94)	(.94)	(.92)	(.92)	(.91)
Eldorado 4101 Ideal6235 156	+5.1	+26	+51	+1.0	-.09	+.09	-.01
#10484440	(.77)	(.74)	(.70)	(.68)	(.64)	(.64)	(.61)
Eileenmere Masterpiece JAO	+4.1	+26	+35	+3.0	-.25	+.08	+.01
#08989782	(.95)	(.95)	(.93)	(.60)	(.58)	(.58)	(.56)
Eldorado 4116 Ideal880 156	+5.9	+19	+49	+8.0	-.16	+.16	+.01
#10484454	(.81)	(.79)	(.73)	(.68)	(.66)	(.66)	(.65)
*Nichols Trademark S1	+4.8	+29	+66	+8.0	-.24	-.17	+.02
#10622930	(.95)	(.95)	(.93)	(.72)	(.68)	(.68)	(.65)
*Rito Excel 809	+1.9	+38	+69	+11	-.18	+.28	+.00
#08781383	(.93)	(.93)	(.91)	(.54)	(.51)	(.51)	(.48)

*Bulls that were selected by Oklahoma State University researchers to supplement EPD data. All EPD values are provided by the National Angus Association 1994 Sire Evaluation. Accuracy values are located in ( ) under the EPD values. BW=birth weight EPD, DWW=direct weaning weight EPD, YW=yearling weight EPD, HCW=carcass weight EPD, MARB=marbling EPD, REA=rib eye area EPD and FAT=fat depth EPD.

Two different slaughter times were designated for steers and heifers in both years. The early slaughter was designed to approximate .25 inches of subcutaneous fat at the 12-13th rib, while the late slaughter was to approximate .50 inches of subcutaneous fat at the 12-13th rib. Early slaughter steers were fed a finishing ration for 124 days, while late kill steers were fed for 191 days. Early slaughter heifers were fed for 85 days, while late slaughter heifers were fed for 148 days. The early and late slaughter times were designed to create variability in external fat thickness beyond that already expected based on sire EPD values. The later of the two slaughter times was designed to be at the industry standard for finished slaughter weight. Measured production traits included birth weight (BW, lbs.) and adjusted weaning weight (AWW, lbs.).

Carcass traits collected were hot carcass weight (HCW, lbs.), rib-eye area (REA, sq. inches), adjusted preliminary yield grade (AFAT) and marbling score (MARB). The rib section (RIB, lbs.) of one half of each carcass was weighed and separated into the ninth-tenth-eleventh section (NTERIB, lbs.) and the remaining rib sections. The ninth-tenth-eleventh rib section was weighed and dissected into subcutaneous fat (NTESC, lbs.), seam fat (NTESM, lbs.), lean (NTELEAN, lbs.), longissimus dorsi muscle (NTEEYE, lbs.) and bone (NTEB, lbs.) weights. We focused on the ninth-tenth-eleventh rib section because it serves as a good indicator of total carcass composition (Hankins and Howe, 1946). The percentage of fat (NTEFAT, %), lean (NTELEN, %) and bone (NTEBN, %) for the NTERIB section was also evaluated for this study. The percentage of fat for the NTERIB was calculated as NTESC + NTESM/ NTERIB. The percentage of lean for the NTERIB was computed as NTELEAN + NTEEYE/ NTERIB. The percentage of bone for the NTERIB was simply NTEB/ NTERIB. In addition to these weights and percentages, Warner-Bratzler shear force (SF) values were collected as well as sensory panel evaluations of flavor (FLAV), muscle fiber tenderness (MFT), connective tissue amount (CT), juiciness (JUC) and overall tenderness (T) on the longissimus dorsi muscle from the ninth-tenth-eleventh rib section. Flavor was evaluated by taste panelists on a 4-

point scale where 4 = no off flavor. The remaining sensory attributes were evaluated on an 8-point scale with 8 being the most desirable (8 = extremely juicy, extremely tender, no connective tissue).

Genotyping

DNA samples were collected for all sires from semen samples (Appendix E) and from all offspring from either blood (Appendix F) or tissue (Appendix G) samples. If necessary, some DNA samples were taken through a short phenol/chloroform clean-up protocol (Appendix H) to improve the quality of the DNA for the Polymerase Chain Reaction (PCR). The concentrations and ingredients for all necessary solutions for DNA extraction protocols can be found in Appendix I. The concentration of all DNA samples were determined using a spectrophotometer (Appendix J). The concentrations of all working solutions of genomic DNA used for PCR were 50 ng/μl.

All animals were genotyped for five loci: kappa-casein (K-Cas), beta-lactoglobulin (B-Lac), growth hormone (GH), pituitary transcription factor (Pit-1) and a polymorphism in a DNA region with high homology to heart-fatty acid binding protein (H-FABP). Genotyping was accomplished through Polymerase Chain Reaction (PCR) amplification of the DNA region of interest and restriction enzyme digestion of the resulting PCR product. Primer sequence information for all PCRs can be found for each of the five genes in Appendix K.

Kappa-Casein

A complete description of the K-Cas polymorphism and methods for PCR genotyping can be found in Medrano and Aguilar-Cordova (1990a). A PCR was done

using the following conditions: 1.5 mM of MgCl, 200 μM of each dNTP (Boehringer-Mannheim, Indianapolis , IN), 0.1 μM of each primer, 50-100 ng of genomic DNA and 0.75 Units of DNA *Taq* polymerase (Boehringer-Mannheim, Indianapolis , IN) in a total volume of 15 μl. The first cycle of the PCR was 2 min at 95°C, 1 min at 60°C and 2 min at 72°C. This was followed by 29 cycles under the conditions of 1 min at 94°C, 1 min at 60°C and 1 min at 72°C. The PCR ended with a 9 min extension phase at 72°C. PCR product (10.68 μl) was digested with 1.25 Units of *Hinf* I (New England BioLabs, Inc., Beverly, MA) for 3 hours at 37°C.

Beta-Lactoglobulin

A complete description of the B-Lac polymorphism can be found in Medrano and Aguilar-Cordova (1990b). The PCR to amplify B-Lac used the following conditions: 1.5 mM of MgCl, 200 μM of each dNTP (Boehringer-Mannheim, Indianapolis, IN), 0.1 μM of each primer, 50-100 ng of genomic DNA and .75 Units of DNA *Taq* polymerase (Boehringer-Mannheim, Indianapolis, IN) in a final volume of 15 μl. The first cycle of the PCR was 2 min at 95°C, 1 min at 60°C and 2 min at 72°C. This was followed by 29 cycles under the conditions of 1 min at 94°C, 1 min at 60°C and 1 min at 72°C. The PCR ended with a 9 min extension phase at 72°C. PCR product (10.68 μl) was digested with 1.25 Units of *Hae* III (New England Biolabs Inc., Beverly, MA) for 3 hours at 37°C.

Growth Hormone

A complete description of the GH polymorphism can be found in Zhang et al. (1992). The PCR reaction conditions to amplify GH were: 1.5 mM of MgCl, 200 μM of each dNTP (Boehringer-Mannheim, Indianapolis, IN), 0.5 μM of each primer, 50-100 ng

of genomic DNA and .50 Units of DNA *Taq* polymerase (Boehringer-Mannheim, Indianapolis, IN) in a final volume of 15 µl. The first cycle of the PCR was 2 min at 95°C, 1 min at 58°C and 2 min at 72°C. This was followed by 30 cycles under the conditions of 1 min at 94°C, 1 min at 58°C and 1 min at 72°C. The PCR ended with a 9 min extension phase at 72°C. PCR product (10.55 µl) was digested with 2.0 Units of *Alu* I (GibcoBRL, Grand Island, NY) for 8 hours at 37°C.

Pituitary Transcription Factor-1

A complete description of the Pit-1 polymorphism can be found in Moody and Pomp (1994). The PCR conditions for amplification of Pit-1 were: 1.5 mM MgCl, 200 µM of each dNTP (Boehringer-Mannheim, Indianapolis, IN), 0.1 µM of each primer, 50-100 ng of genomic DNA and .50 Units of DNA *Taq* polymerase (Boehringer-Mannheim, Indianapolis, IN) in a final volume of 15 µl. The first cycle of the PCR was 2 min at 95°C, 1 min at 55°C and 2 min at 72°C. This was followed by 29 cycles under the conditions of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C. The PCR ended with a 9 min extension phase at 72°C. PCR product (10.68 µl) was digested with 1.25 Units of *Hinf* I (New England Biolabs Inc., Beverly, MA) for 3 hours at 37°C.

Development of New DNA Markers

The development of a PCR to amplify regions of the bovine genomic sequences for LPL and FABP were also attempted. This process began with the designing of PCR primers based on available bovine cDNA sequences for the respective genes. A bovine cDNA sequence for LPL was published by Senda et al. (1987) and for bovine heart-FABP by Billich et at. (1988). A standard optimization for newly developed primers using

varying concentrations of MgCl, dNTPs (Boehringer-Mannheim, Indianapolis, IN) and primers was done beginning with an annealing temperature of 55° C. If several bands of PCR product were produced at this annealing temperature, then the annealing temperature was raised a few degrees. The annealing temperature was increased if necessary (65° C for LPL) to produce a specific band of PCR product when run on a 1% agarose gel. Once the PCR conditions were successful in amplifying only one product consistently that was the expected size, then restriction enzyme digests were examined for polymorphisms. The number of cycles of the PCR was also increased if the normal 30 cycle PCR length produced faint products. The PCR for amplification of the H-FABP product had a cycle number of 35 for this reason.

A bovine panel was established that consisted of Hereford (n=2), Holstein (n=2), Simmental (n=2), Limousin (n=2), Brangus (n=2), Angus (n=2), Brahman (n=4) and Gelbvieh (n=2) cattle. Genomic DNA for these 18 animals was amplified and then digested with restriction enzymes to attempt to identify any restriction fragment length polymorphisms (RFLP) that may exist. The RFLP patterns were visualized on 3% agarose gels. With LPL, an RFLP was detected using the bovine panel with the restriction enzyme *Sau* 96 I (New England Biolabs Inc., Beverly, MA). A complete description of this polymorphism can be found in Appendix L.

The PCR product produced with primers designed from the bovine H-FABP cDNA sequence was the exact size (612 bp) as the cDNA. Sequence data was attained for the entire 612 bp of the PCR product. This sequence data revealed a high degree of homology to the bovine H-FABP cDNA and to the bovine mammary derived growth inhibitor (MDGI) cDNA. However, the PCR product was not a perfect match for either of these two cDNA sequences. This suggests that there is a high probability that this PCR amplification product is a pseudogene for either the H-FABP or MDGI genes. Due to the lack of information about the true identity of this PCR product, it will be referred to in this text as simply the H-FABP marker. Additional research will be required before the

identity of this PCR product will be known. A polymorphism with *Rsa* I (GibcoBRL, Grand Island, NY) was detected in the H-FABP amplification product. A complete description of this polymorphism is found in Appendix M. PCR conditions for amplification of the H-FABP marker were: 0.75 mM MgCl, 100 μM of each dNTP (Boehringer-Mannheim, Indianapolis, IN), 0.1 μM of each primer, 0.42 Units of *Taq* DNA polymerase (Boehringer-Mannheim, Indianapolis, IN) and 50 - 100 ng of genomic DNA in a total volume of 12 μl. The first cycle of the PCR was 2 min at 95°C, 1 min at 55°C and 2 min at 72°C. This was followed by 34 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C. The PCR ended with a 9 min extension phase at 72°C. PCR product (10.55 μl) was digested with 2.5 Units of *Rsa I* (GibcoBRL, Grand Island, NY) at 37°C for 8 hours.

Single stranded conformation polymorphisms (SSCP) provide another opportunity to search for polymorphisms within the DNA sequence. The procedure used for all SSCP work can be found in Appendix N. An SSCP was found using the H-FABP marker PCR amplification product (data not shown), however this SSCP was polymorphic for the same animals as the PCR based RFLP using *Rsa* I (GibcoBRL, Grand Island, NY) that was described earlier. Due to its ease of use, the PCR-RFLP was used instead of the SSCP for this genetic marker study. No SSCP was identified for the LPL PCR product.

Allele Visualization

Digestion products were electrophoresed on a 3% agarose gel (Appendix I) for 1 hour at 73 volts. The agarose gel was stained for 30 minutes in ethidium bromide (5 μ g/ml; Sigma Chemical Company, St. Louis, MO) and destained for 30 min in dd$H_2$0. The different alleles for all five genes were visualized by viewing under ultraviolet light. Two alleles (A&B) were identified for all genes evaluated. Each animal was assigned a genotype of AA, AB, or BB.

Statistical Analysis

Allele Frequency Analyses

Once genotypes were obtained for all sires and offspring, allele (A&B) frequencies were calculated within the high and low marbling groups for each of the five loci. Allele frequencies were calculated for the A allele as 2(AA) + AB/ 2N where N equals the total number of animals genotyped in the respective marbling group. The B allele frequency was calculated as 2(BB) + AB/ 2N. A Chi-square test was used to identify any significant differences between the high and low marbling groups for both sire and offspring allele frequencies. The offspring population was also tested using the Chi-square statistic for agreement with the Hardy-Weinberg Law.

Genotype Effect on Phenotype: Least-Squares Analysis

Effect of genotype on the measured production and carcass characteristics was tested using two types of statistical methods. Least-squares analysis was performed to test for significant genotype effects on all production and carcass traits. Growth traits were analyzed including effects of sire, genotype, year and sex as well as all two and three way interactions between year, sex and genotype. The model was then simplified for ease of computation through the removal of insignificant ($P>.20$) interaction terms and the formation of a composite variable for year and sex (YS). The final model for least-squares analysis of BW and AWW included effects of sire, genotype, a fixed-effects composite variable called YS (Year-Sex), the YS x genotype interaction and residual. The model used for least-squares analysis of all carcass traits included effects of sire, genotype, slaughter time, year and sex as well as all two, three and four way interactions between

genotype, slaughter time, year and sex. A simplified model was developed that removed insignificant interactions. The revised model included effects of sire, genotype, a fixed-effects composite variable called TYS (Slaughter Time-Year-Sex) and the TYS x genotype interaction and residual. This model was successful for generating least-squares means for all genotype classifications for the evaluated loci except for K-Cas and B-Lac. Additional reduction of the model was needed to generate least-squares means for these two loci due to poor genotypic distributions that were causing missing data in interaction classes. Removal of the four-way interaction of slaughter time x year x sex x genotype from the revised model produced least-squares means for the K-Cas genotypes. Removal of the four-way interaction term, as well as all three way interaction terms was necessary to produce the least-squares means for B-Lac genotypes.

Contrasts were performed using least-squares analyses with the models described above minus the composite variable x genotype interaction term. Contrast 1 was designed to test for dominance gene action and was set up as C1= AA + BB - 2(AB). Contrast 2 was designed to test for additive gene action and was set up as C2= AA - BB. The significance of the dominance contrast does indicate dominant gene action, however, it is possible for both the additive and the dominant contrasts to be significant when there is dominant gene action. The additive contrast ignores the presence of dominant gene action.

The percentage of total and residual variation explained by the genotype of interest was also calculated to provide an indication of how good a job each genotype was doing at explaining the variation in the traits it significantly affected. The percentage of total variation explained by genotype was calculated as the sum of squares for genotype divided by the corrected total sum of squares. The percentage of residual variation explained by genotype was computed as the genotype sum of squares divided by the corrected total sum of squares minus the variation explained by sire and the composite variable TYS.

Genotype Effects on Phenotype: Mixed Model Analysis

A mixed model analysis was performed under a sire model with Multiple Trait Derivative-Free Restricted Maximum Likelihood (MTDF-REML; Boldman, 1993) to test for genotype significance on all production and carcass traits. The model used to analyze BW and AWW was Trait = Year + Sex + Genotype + Sire + Residual. The model used to analyze the remaining carcass traits was Trait = TYS + Genotype + Sire + Residual. The traits for shear force and sensory panel values were not evaluated in the mixed model analysis. All traits analyzed had homogenous variances except for MARB. There was a much larger variance in the late slaughter time for both steers and heifers than in the early slaughter time. Therefore, the data for the early slaughter was adjusted to have the same variance as the late slaughter which should be closest to industry data. Linear contrasts determined if gene action was dominant or additive in nature. Contrast 1= AA + BB - 2(AB) was used to identify dominant gene action, while contrast 2 = AA - BB was used to identify additive gene action. The significance of the additive contrast does not necessarily suggest additive gene action since this contrast ignores the presence of dominant gene action. It is possible for both the additive and the dominance contrasts to be significant at the same time. If both contrasts are significant it would suggest the presence of dominant gene action.

The heritability values and sire variance components used for the mixed model analyses are presented in Table 5. The heritability values are based on data presented in the American Angus Association's sire summary (1994) and data presented by Marshall (1993). The sire variance values were generated through the mixed model analysis. The heritability values are used in the mixed model analysis to account for the relationship between genotype and phenotype. The sire variance components are needed to adjust sire

effects. If sire variance components were not used, we would then be treating sire effects as if they were fixed effects, which is not the case.

Sire Regression Analysis

A regression analysis of EPD value on genotype was done for all sixteen purebred Angus sires. The EPD values evaluated included: birth weight (BW), direct weaning weight (DWW), yearling weight (YW), hot carcass weight (HCW), marbling score (MARB), rib-eye area (REA) and fat thickness (FAT). A separate evaluation was done for each of the five loci: K-Cas, B-Lac, GH, Pit-1, and H-FABP. Within the regression analysis the AA genotype was assigned a value of 2, the AB genotype a value of 1 and the BB genotype a value of 0. The EPD values used for all evaluated traits can be found in Table 3 and Table 4.

Table 5. Heritability and Sire Variance Components for Mixed Model Analyses

TRAIT	h^2	Sire Variance
BW	.39	10.3
AWW	.28	101.3
HCW	.30	227.2
AFAT	.25	.00092
REA	.30	.109
MARB	.28	568
RIB	.30	.556
NTERIB	.30	.2215
NTELEAN	.30	.0292
NTEEYE	.30	.0099
NTESC	.25	.0110
NTESM	.30	.0395
NTEB	.50	.0055

CHAPTER IV

RESULTS

Allele Frequency Data

Allele frequencies (A & B) were calculated for each of the five evaluated loci for the twelve Angus sires in the low marbling (LM) and high marbling (HM) groups. The sire allele frequencies are presented in Table 6. Allele frequencies for the 240 offspring in the low marbling (LM) and high marbling (HM) groups were also calculated. These allele frequencies can be found in Figure 1 (K-Cas), Figure 2 (B-Lac), Figure 3 (GH), Figure 4 (Pit-1) and Figure 5 (H-FABP).

Table 6. Allele Frequencies in High Marbling (HM) and Low Marbling (LM) Sires for Five Evaluated Loci

LOCI	H M		L M	
	A	B	A	B
K-Cas	.83	.17	.83	.17
B-Lac*	0	1.0	.25	.75
GH*	.92	.08	.58	.42
Pit-1	.50	.50	.33	.67
H-FABP	.83	.17	1.0	0

LM = Low marbling sire group; HM = High marbling sire group. Loci with an * following them have significantly different (P<.10) allele frequencies between LM and HM sire groups.

Sire allele frequencies between HM and LM groups were tested for significant differences with a Chi-square test. The allele frequencies were significantly different (P<.10) between the HM and LM sire groups for B-Lac and GH. Allele frequencies were

not significantly different (P>.10) for K-Cas, Pit-1 and H-FABP. The allele frequencies in all 240 offspring should reflect any differences in allele frequencies which were identified in the sire groups. Significant differences in allele frequencies that are detected between the offspring HM and LM groups that were not detected in the sire allele frequencies could result from the increased statistical power resulting from the larger population size or from dam alleles.

A chi-square test was performed to test for any significant differences between allele frequencies for the high marbling and low marbling offspring groups. Significant differences (P<.01) were detected for B-Lac, GH and the H-FABP loci. K-Cas and Pit-1 allele frequencies were not significantly different (P>.10) between the high and low marbling offspring groups.

All five loci were tested to see if they were in Hardy-Weinberg Equilibrium (data not shown). The Hardy-Weinberg law states that in a large randomly mating population, if there is no migration and mutation can be safely ignored, then gene and genotypic frequencies are constant from generation to generation. The chi-square test showed no significant deviations for any of the five tested loci from Hardy-Weinberg Equilibrium within this population.

Genotypic distributions for each of the five loci are presented in Table 7. These numbers represent the total number of animals genotyped for each locus, as well as the distribution of genotypes for each gene. Notice should be taken of the low number of animals in certain genotype categories, such as the BB homozygote for K-Cas, the AA homozygote for B-Lac and the complete lack of the BB homozygote genotype for the H-FABP locus. A few animals with extreme values in these genotype categories could drastically influence the means calculated for that specific genotype.

Figure 1. Allele Frequencies in High Marbling (HM) and Low Marbling (LM) Offspring Groups for Kappa-Casein

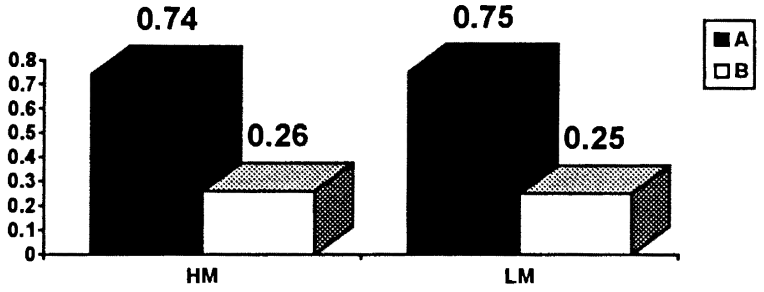


Figure 2. Allele Frequencies in High Marbling (HM) and Low Marbling (LM) Offspring Groups for Beta-Lactoglobulin

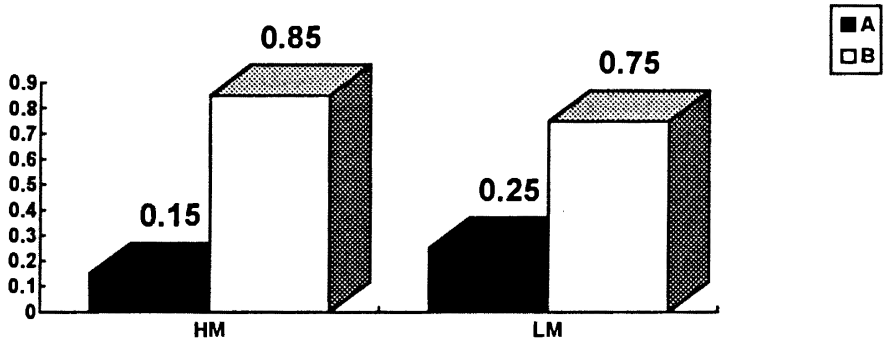


Figure 3. Allele Frequencies in High Marbling (HM) and Low Marbling (LM) Offspring Groups for Growth Hormone

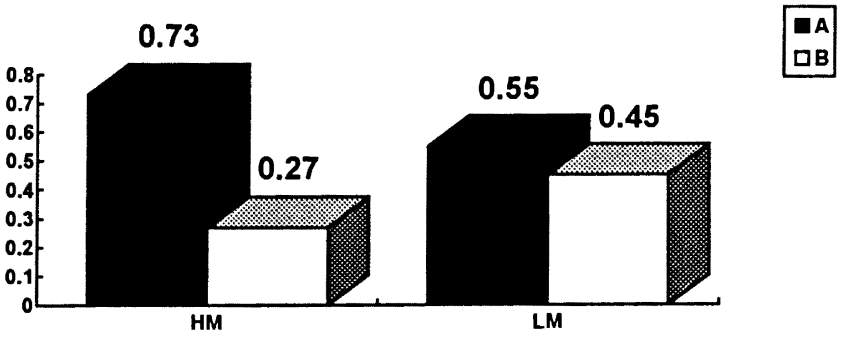


Figure 4. Allele Frequencies in High Marbling (HM) and Low Marbling (LM) Offspring Groups for Pituitary Transcription Factor-1

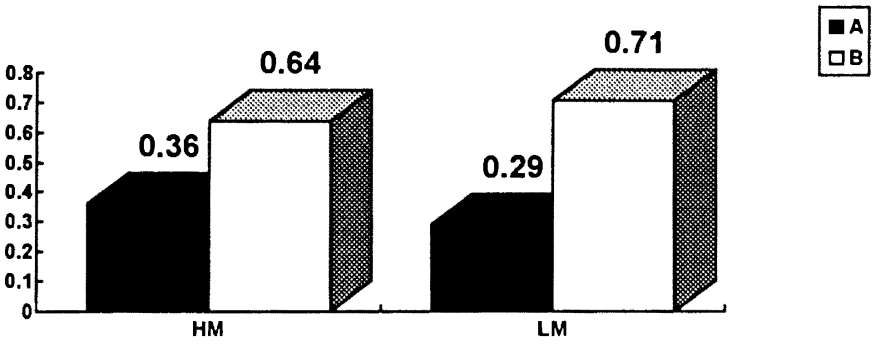


Figure 5. Allele Frequencies in High Marbling (HM) and Low Marbling (LM) Offspring Groups for Heart-Fatty Acid Binding Protein

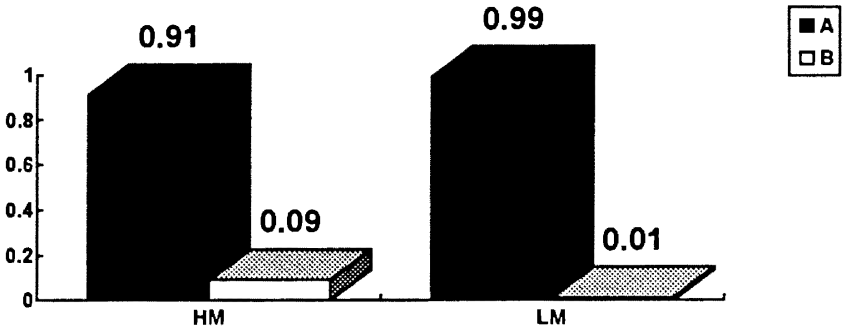


Table 7. Genotypic Distributions for All Five Evaluated Loci

Genotype	K-CAS	B-LAC	GH	PIT-1	H-FABP
AA	133	11	94	28	216
AB	92	76	116	95	24
BB	15	153	29	112	0
TOTAL	240	240	239	235	240

Least-Squares Analysis

The significant main effects for genotype that were detected using the least-squares analysis are presented in Table 8. All of the traits listed in Table 8 had significant effects of sire (except for Pit-1 genotype on NTEB and GH genotype on SF). The composite variable TYS (YS for AWW) was also significant for all traits listed in Table 8. Table 9 presents contrast results for all traits identified to be significantly effected by genotype through least-squares analysis. These contrast results were used to indicate if gene action on the trait was additive or dominant in nature. Least-squares means are presented for all traits listed in Table 8 for each gene found to have significant effects. Least-squares means are in Table 10 (K-Cas), Table 11 (GH) and Table 12 (Pit-1). The least-squares means for B-Lac genotype on RIB weight and H-FABP genotype on AWW will be described within their respective sections of this text.

Table 8. Significant Genotype Effects Detected using Least-Squares Analysis

K-CAS	B-LAC	GH	PIT-1	H-FABP
HCW**	RIB+	HCW*	HCW+	AWW+
AFAT*		RIB*	RIB*	
REA**		NTESM+	NTERIB*	
RIB*		NTEEYE+	NTESC**	
NTERIB+		SF**	NTESM*	
NTESC+			NTEB**	
NTEEYE**			NTEFAT*	
NTEFAT+			NTELEN*	
NTEBN+			NTEBN*	

+ P<.10, * P<.05, ** P<.01

Table 9. Additive and Dominant Contrast Results for Traits Significantly Affected by Genotype

TRAIT	K-CAS	B-LAC	GH	PIT-1
HCW	**B****		**D+**	**A***
RIB	**D***	**B***	NS	**A****
REA	**B****			
AFAT	NS			
NTERIB	**A+**			**A****
NTESC	**A***			
NTESM			NS	**A****
NTEEYE	**B***		**D+**	
NTEB				**D****
NTEFAT	NS			**A+**
NTEBN	NS			NS
NTELEN				NS
SF			**A****	

+ P<.10, * P<.05, ** P<.01, NS = Neither contrast is significant. A = Additive Gene Action, D = Dominant Gene Action, B = Both Contrasts are Significant. No contrasts were done for H-FABP due to the lack of animals with the BB genotype.

Table 10. Least-Squares Means for Kappa-Casein

TRAIT	AA	AB	BB
HCW*	682 ± 4.8^{a}	684 ± 5.6^{a}	636 ± 20.7^{b}
AFAT*	$3.13 \pm .03^{a}$	$3.02 \pm .03^{b}$	$2.94 \pm .12^{a,b}$
REA**	$12.24 \pm .10^{a}$	$12.30 \pm .11^{a}$	$10.97 \pm .43^{b}$
RIB**	$29.5 \pm .24^{a}$	$29.8 \pm .28^{a}$	26.9 ± 1.05^{b}
NTERIB*	$11.84 \pm .11^{a}$	$11.76 \pm .13^{a}$	$10.78 \pm .48^{b}$
NTESC*	$1.39 \pm .03^{a}$	$1.32 \pm .03^{a,b}$	$1.13 \pm .12^{b}$
NTEEYE**	$2.02 \pm .02^{a}$	$1.99 \pm .03^{a}$	$1.70 \pm .10^{b}$
NTEFAT*	$.33 \pm .003^{a}$	$.32 \pm .004^{b}$	$.32 \pm .01^{a,b}$
NTEBN*	$.18 \pm .002^{a}$	$.19 \pm .002^{b}$	$.19 \pm .006^{a,b}$

All values with similar superscripts in the same row are not significantly different (P>.10) and values with different superscripts in the same row are significantly different with level of significance designated by * (P<.05) or ** (P<.01) following the trait abbreviation.

Table 11. Least-Squares Means for Growth Hormone

TRAIT	AA	AB	BB
HCW**	668 ± 5.6^{a}	690 ± 5.0^{b}	$688 \pm 12^{a,b}$
RIB**	$29.0 \pm .28^{a}$	$30.1 \pm .25^{b}$	$29.8 \pm .60^{a,b}$
NTESM*	$2.49 \pm .06^{a}$	$2.65 \pm .05^{b}$	$2.45 \pm .12^{a,b}$
NTEEYE*	$1.94 \pm .03^{a}$	$2.03 \pm .03^{b}$	$2.03 \pm .06^{a,b}$
SF**	$2.57 \pm .05^{a}$	$2.62 \pm .05^{a}$	$2.97 \pm .12^{b}$

All values with similar superscripts in the same row are not significantly different (P>.10) and values with different superscripts in the same row are significantly different with level of significance designated by * (P<.05) or ** (P<.01) following the trait abbreviation..

Table 12. Least-Squares Means for Pituitary Transcription Factor-1

TRAIT	AA	AB	BB
$HCW^{+,*}$	704 ± 12.8^{a}	$681 \pm 5.6^{b(+)}$	$675 \pm 5.2^{b(*)}$
RIB**	$31.2 \pm .63^{a}$	$29.5 \pm .27^{b}$	$29.2 \pm .26^{b}$
NTERIB**	$12.5 \pm .29^{a}$	$11.7 \pm .13^{b}$	$11.6 \pm .12^{b}$
NTESC**	$1.59 \pm .08^{a}$	$1.33 \pm .03^{b}$	$1.32 \pm .03^{b}$
NTESM*	$2.84 \pm .13^{a}$	$2.56 \pm .06^{b}$	$2.48 \pm .05^{b}$
NTEB**	$2.29 \pm .06^{a}$	$2.11 \pm .03^{b}$	$2.20 \pm .02^{a}$
$NTEFAT^{*,+}$	$.35 \pm .01^{a}$	$.33 \pm .004^{b(+)}$	$.32 \pm .004^{b(*)}$
$NTELEN^{*,**}$	$.46 \pm .008^{a}$	$.49 \pm .004^{b(**)}$	$.48 \pm .003^{b(*)}$
NTEBN*	$.18 \pm .006^{a,b}$	$.18 \pm .002^{a}$	$.19 \pm .002^{b}$

All values with similar superscripts in the same row are not significantly different (P>.10) and values with different superscripts in the same row are significantly different with level of significance designated by $^{+}$ (P<.10), * (P<.05) or ** (P<.01) following the trait abbreviation.

Kappa-Casein

K-Cas genotype had significant influences on several carcass traits with the most notable effects on HCW, REA and NTEEYE. Main effects of sire and TYS were significant for all listed traits in Table 8 at the $P \leq .01$ level for K-Cas analyses, except for NTEBN. Sire was significant at $P < .10$ for K-Cas effect on NTEBN. The TYS x K-Cas interaction term was significant at $P < .10$ for REA and at $P < .05$ for NTEEYE.

The HCW least-squares means figured for K-Cas genotype yielded significant additive and dominant contrasts. This is easily explained when evaluating the least-squares means for HCW. The AA and AB genotypes are not significantly different ($P > .10$) in their mean values while the BB genotype has a mean value for HCW approximately 50 pounds lighter then the other two genotypes. This large difference between the two homozygote genotypes would yield the additive contrast significant. The slightly higher value of the heterozygote and the low value for the BB genotype would cause a significant dominance contrast. Through evaluation of the contrast results and the least-squares means it appears that the A allele for K-Cas is dominant for heavier carcass weights, yielding an increase of 45-50 pounds in HCW over the mean of the BB homozygote. The traits of REA and NTEEYE also have both additive and dominant contrasts significant, and appear to have the same allele effects as witnessed on HCW by K-Cas genotype. Once again, the A allele tends to act in a dominant manner with the AA and AB genotypes having REA measurements of approximately 12 square inches. The BB genotype in comparison has a significantly lower ($P < .01$) mean value at 10.9 square inches of REA. The least-squares means for NTEEYE depict the same type of gene action, with the A allele yielding a significant ($P < .01$) increase of three-tenths of a pound in NTEEYE weight over the BB genotype least-squares mean of $1.70 \pm .10$. The trait RIB was significant for only the dominance contrast. The dominant gene action of K-Cas

is evidenced by the fact that the AA and AB genotypes have RIB weights around 29.5 pounds, while the BB genotype for K-Cas has a least-squares mean for RIB of 26.9. The least-squares means for NTERIB indicate complete dominance of the A allele, even though the dominance contrast was not significant and the additive contrast is significant. The effect of the K-Cas A allele on NTESC weight is also dominance. The AB genotype has a least-squares mean that is intermediate to the two homozygous means, however, the heterozygote mean for NTESC is closer to the AA mean than to the BB mean. Mean values such as these would yield the dominance contrast not significant, while still resulting in a significant additive contrast. The A allele for both NTERIB and NTESC yields an increase in weights for both of these traits. The BB genotype still possesses the lowest weight for NTERIB and for NTESC. Although the AB and AA genotypes for K-Cas were significantly different ($P<.05$) for AFAT, there were no significant contrasts identified. The least-squares means for AFAT would suggest the additive gene action of K-Cas genotype. Considering the fact that the AFAT measurement was taken as an adjusted preliminary yield grade, it would appear that there is not enough variation between the three genotypes to identify one of the two contrasts as significant. The fact that the AA and AB genotypes were significantly different ($P<.05$), and that the AA and BB genotypes approached being significantly different ($P<.11$) would indicate that the affect of the A allele to increase fat as indicated for NTESC is still represented for AFAT. The least-squares means for NTEFAT indicate that AA genotype has the highest amount of fat as a percentage of NTERIB weight. The percentage of bone (NTEBN) is highest for the AB and BB genotypes of K-Cas. Neither of the two contrasts were significant for NTEFAT or NTEBN.

Beta-Lactoglobulin

The main effects of sire and the composite variable TYS ($P<.01$) were significant in addition to B-Lac genotype ($P<.10$) for RIB weight. The interaction term of TYS x B-Lac was not significant ($P>.10$) for the RIB weight analysis.

The AA genotype for B-Lac had a RIB weight of $32.07 \pm .96$, while the AB genotype had a least-squares mean value for RIB weight of $29.5 \pm .33$ and the BB genotype had a value for rib weight of $29.3 \pm .22$. The AA homozygote value is significantly different ($P<.01$) from both the AB and BB genotype least-squares means. The AB and BB genotype least-squares means were not significantly different ($P>.10$) from one another. Both contrasts were significant ($P<.05$) for the effect of B-Lac on RIB weight. This can be explained due to the large difference between the two homozygote genotypes (causing a significant additive contrast), and the appearance of dominant gene action since the AB and BB genotypes were not significantly different ($P>.10$). It would appear from evaluation of the least-squares means that the B allele is dominant yielding a decrease in RIB weight on average of 1.5-2.0 pounds when compared to the higher RIB weight of the AA genotype.

Growth Hormone

GH genotype had significant effects on carcass and subprimal weights as well as having significant effects on NTESM and NTEEYE. The main effects of sire and TYS were significant ($P<.01$) for HCW, RIB and NTEEYE. NTESM had a significant main effect of sire and TYS x GH interaction term at the $P<.05$ level and a significant effect of TYS ($P<.01$). The main effect of sire was not significant for SF, however the TYS and TYS x GH interaction term were both significant at the $P<.01$ level.

Two significant contrasts for GH detected dominant gene action on HCW and NTEEYE. When evaluating the least-squares means for GH genotype on HCW the AB genotype is significantly different from the AA genotype for HCW. However, the BB genotype which is intermediate in value between the AA and AB genotype is not significantly different from either of these two genotypes. This would suggest dominance of the B allele. The BB genotype has a least-squares mean that is not significantly different from the heterozygote mean. The B allele tends to yield an increase in carcass weight. The least-squares means for NTEEYE indicate a significant complete dominance effect of the B allele for increased NTEEYE weights. Once again, the AA genotype has the lowest NTEEYE weight. Neither contrast was identified as significant for the traits of RIB and NTESM. In both of these cases, the heterozygote has the highest mean for RIB and NTESM, and is always significantly different from the AA genotype mean. The GH genotype was significant for Warner-Bratzler shear force values. The least-squares means indicate the dominance of the A allele of GH for lower shear force values. The significant contrast was additive, however there was no significant difference between the AA and AB genotypes for shear force values.

Pituitary Transcription Factor-1

The Pit-1 genotype affected several carcass traits which included carcass and subprimal weights, as well as fat. Sire was significant ($P \leq .01$) for all traits listed in Table 8 with significant main effects for Pit-1, except for NTEB and NTEBN. The composite variable TYS was significant ($P < .01$) for all traits significant for Pit-1 genotype. The interaction term of TYS x Pit-1 was significant at the $P < .10$ level for RIB and NTESC.

Significant contrasts identified additive gene action of Pit-1 genotype on HCW, RIB, NTERIB and NTESM. In all of these cases, the heterozygote value is intermediate

to the two homozygotes, however, the AB genotype is never significantly different (P>.10) than the BB genotype least-squares means. This indicates the incomplete dominance of the B allele of Pit-1. In all of these situations the AA genotype has the highest values for carcass, subprimal and fat weights. A significant dominant contrast was detected for Pit-1 genotype on NTEB. The trait NTEB is affected differently by Pit-1 genotype then any of the other traits. The dominance contrast was significant for this trait, even though there does not appear to be any dominant gene action according to the least-squares means. The least-squares mean for the AB genotype for NTEB is significantly different from either of the two homozygotes. When evaluating the traits that were percentages of the NTERIB weight for fat, lean and bone, all were significant for Pit-1 genotype. Although the significant contrast was additive for NTEFAT, the least-squares means indicate the complete dominance of the B allele for less fat as a percentage of the NTERIB. The B allele also tended to yield an increase in the percentage of lean. The BB genotype had the highest percentage of bone, however, it was not significantly different from the AA genotype percentage.

Heart-Fatty Acid Binding Protein Marker

Significant main effects of H-FABP genotype (P<.10) sire and the YS composite variable (P<.01) were detected for AWW with the least-squares analysis. Due to the lack of animals in the BB genotype category for the H-FABP marker, there were no contrast to indicate gene action of this marker. The AA genotype had a least-squares mean for AWW of 516.8 $\pm$ 2.6, while the AB genotype group had a least-squares mean for AWW of 533.1 $\pm$ 8.6. The least-square means for the AA and AB genotype groups for AWW were significantly different at P<.10.

Variation Table Results

The percentage of total and residual variation explained by genotype for those traits significantly effected by genotype can be found in Table 13. The traits of REA and NTEEYE have the highest percentage of total variation within that trait explained by K-Cas genotype. However, when looking at the percentage of residual variation, K-Cas explains a similar percentage of trait variation for HCW, REA and NTEEYE. The percentage of total variation explained by GH genotype is similar for all traits listed in Table 13, however, the traits of HCW and RIB do have higher percentages of residual variation explained by GH genotype than NTESM and NTEEYE. Two traits stand out when looking at the total and residual percentages of variation explained by Pit-1 genotype. These two traits are NTESC and NTEB. Both of these traits have noticeably higher values than the other traits listed in Table 13. The percentage of total (.69%) and residual (2.0%) variation explained by B-Lac genotype for RIB weight is not extremely high. The percentage of total (.95%) and residual (1.4%) variation explained by the H-FABP marker for AWW are also not out of the ordinary.

Mixed Model Analysis

The results for contrasts done using the mixed model analysis (Sire Model) are presented in Table 14. The contrast results attained from the mixed model analysis are very consistent with the results from the least-squares analysis. The mixed model analysis would routinely set one genotype value at zero and then express the means of the other two genotypes as deviations from the genotype mean that was set as zero. These deviations from the genotype set as zero allow us to determine the affect that each allele is

Table 13. Total and Residual Variation (Sums of Squares) Explained by Genotype for Traits Significantly Affected by Genotype

GENE	TRAIT	% TOTAL	% RESIDUAL
K-Cas	HCW	1.4	4.3
K-Cas	AFAT	1.6	2.9
K-Cas	REA	2.3	4.9
K-Cas	RIB	1.2	3.5
K-Cas	NTERIB	0.94	2.1
K-Cas	NTESC	1.3	2.6
K-Cas	NTEEYE	2.0	4.2
K-Cas	NTEFAT	1.1	2.1
K-Cas	NTEBN	1.7	2.4
GH	HCW	1.3	4.1
GH	RIB	1.2	3.5
GH	NTESM	1.2	2.5
GH	NTEEYE	1.3	2.7
GH	SF	2.0	3.7
Pit-1	HCW	0.66	2.0
Pit-1	RIB	1.2	3.6
Pit-1	NTERIB	1.5	3.5
Pit-1	NTESC	2.5	4.8
Pit-1	NTESM	1.5	3.1
Pit-1	NTEB	3.6	4.8
Pit-1	NTEFAT	1.4	2.7
Pit-1	NTELEN	1.8	3.3
Pit-1	NTEBN	1.9	2.8

having on the value of the effected trait. The deviations from the BB homozygote (set at zero) for significant contrast results are presented for K-Cas (Table 15), GH (Table 16) and Pit-1 (Table 17). There were no significant contrasts identified for the H-FABP loci for all traits evaluated.

Table 14. Additive and Dominant Contrasts Results for Mixed Model Analysis

TRAIT	K-CAS	B-LAC	GH	PIT-1
HCW	**B***	NS	**D***	**A**$^{+}$
REA	**B***	NS	**D**$^{+}$	NS
MARB	NS	NS	**A**$^{+}$	NS
AFAT	NS	NS	**D****	NS
RIB	**D***	**B**$^{+}$	**D**$^{+}$	**A***
NTERIB	NS	NS	NS	**A**$^{+}$
NTESM	NS	NS	NS	**A**$^{+}$
NTEEYE	**A**$^{+}$	NS	NS	NS

+ P<.10; * P<.05; ** P<.01; NS = No significant contrasts; B = Both contrasts are significant; A = The additive contrast is significant; D = The dominant contrast is significant.

Table 15. Genotype Deviations from BB Homozygote Means for Traits with Significant Contrasts for Kappa-Casein

TRAIT	AA	AB	BB
HCW	39.0	44.9	0
REA	.76	.88	0
RIB	1.32	1.81	0
NTEEYE	.19	.18	0

Table 16. Genotype Deviations from the BB Homozygote Means for Traits with Significant Contrasts for Growth Hormone

TRAIT	AA	AB	BB
HCW	-11.4	10.6	0
REA	-.15	-.04	0
MARB	37.6	37.5	0
AFAT	-.05	-.02	0
RIB	-.59	.37	0

Table 17. Genotype Deviations from the BB Homozygote Means for Traits with Significant Contrasts for Pituitary Transcription Factor-1

TRAIT	AA	AB	BB
HCW	20.8	1.1	0
RIB	1.32	.13	0
NTERIB	.69	-.01	0
NTESM	.31	.09	0

Kappa-Casein

The contrasts express varying gene action of K-Cas on the traits it significantly effects. The traits of HCW and REA have both contrasts significant, while RIB is influenced by dominant gene action of K-Cas. These findings are perfect matches for the least-squares contrast results for K-Cas. However, the trait NTEEYE had both contrasts significant for the least-squares analysis, while the mixed model analysis found only the additive contrast to be significant. The traits of HCW, REA and RIB all had the heterozygote with the highest positive deviation from the BB homozygote. The trait NTEEYE expressed little difference between the AA and AB genotypes, with the AA genotype having a slightly larger deviation than the AB genotype from the BB mean.

Beta-Lactoglobulin

The action of B-Lac on RIB weight expresses both additive and dominant gene action. This confirms the contrast results found using the least-squares analysis. The AA genotype for B-Lac had a deviation from the homozygote BB mean for rib weight of 1.63 pounds, while the AB genotype had a deviation of -.07 pounds from the BB genotype mean. The BB genotype was set at 0 deviations. Therefore, the AA genotype of B-Lac tends to have the heaviest RIB weights.

Growth Hormone

The predominant effect of GH genotype on most carcass traits would appear to be dominant gene action. The only deviation from this finding would be the additive gene action of GH on marbling score. The trait HCW has the largest positive deviation for the heterozygote, with the homozygote BB intermediate between the AA and AB genotypes. The AA homozygote appears to have the smallest REA, while the BB genotype has the largest REA. The AA and AB genotypes of GH would appear to have higher marbling scores than the BB genotype. The AA and AB genotypes have slightly lower adjusted preliminary yield grades (AFAT) than the BB homozygote. The AB heterozygote has the highest RIB weight, while the AA homozygote would have the lowest RIB weight for GH genotypes.

Pituitary Transcription Factor-1

The action of Pit-1 genotype on traits with significant contrast values was additive in all cases. The AA homozygote for Pit-1 genotype appears to have the heaviest HCW, RIB and NTERIB weights, as well as the most seam fat (NTESM). The heterozygote value for Pit-1 genotype is only slightly above the BB genotype mean for all traits except for NTERIB. In the case of NTERIB, the AB genotype is slightly below the BB mean.

Sire Regression Analysis

Regression analysis was performed to see if genotypes of evaluated loci could explain variation in sire EPD values for traits of interest. Some significant associations were identified and are presented. Table 18 identifies traits that were significantly effected

by genotype and the resulting R^2 values. The GH genotype is significant ($P<.10$) for explaining approximately 22% of the variation in both marbling and fat thickness EPD values for the sixteen Angus sires. The Pit-1 genotype is significant ($P<.05$) for explaining 27% and 32% respectively of the variation in direct weaning weight and yearling weight EPDs for these sires. The H-FABP genotype would appear to explain a large amount (65%) of the variation in birth weight EPD and approximately 20% of the variation in carcass weight and marbling EPDs. However, the significance of the EPD values explained by the H-FABP genotype should be closely evaluated due to the fact that only two sires in the low marbling group possessed the AB heterozygote genotype, all other sires were homozygous AA.

Table 19 represents the expected EPD values obtained using the regression equation and the observed average EPD values for each of the three genotype groups for those traits that were significant. The predicted values for EPDs influenced by GH and Pit-1 genotypes match quite closely to the observed EPD values for the three genotype classifications. The least accurate predictions of EPD values based on the regression equations when compared to actual observed values are found for birth weight and carcass weight EPDs when predicted by H-FABP genotype.

Figure 6 represents the regression line for marbling EPD on GH genotype, Figure 7 represents the regression line for fat thickness EPD on GH genotype. In both cases, there were no sires with the BB genotype for GH. Therefore, the observed line only runs from the AA to the AB genotype. However, in both cases for the expected marbling and fat thickness EPDs calculated from the regression equation the expected line falls directly on top of the observed line for the AA and AB genotypes.

The regression lines for weaning weight and yearling weight EPD values on Pit-1 genotype are in Figures 8 and 9, respectively. In both of these graphs, we see almost exact matches for the expected and observed lines for the AB and BB genotypes. The

largest deviation from the expected and observed values occurs at the AA homozygote value.

Figures 10, 11 and 12 have regression lines for birth weight, carcass weight and marbling EPDs on H-FABP genotype, respectively. The expected and observed lines are closest at the AA homozygote and tend to deviate the most from one another at the BB homozygote values for both birth weight and carcass weight EPDs. The H-FABP genotype appears to do the best job predicting marbling EPD values based on the similarity between the expected and observed lines shown in Figure 12.

Table 18. Results from Sire Regression Analysis

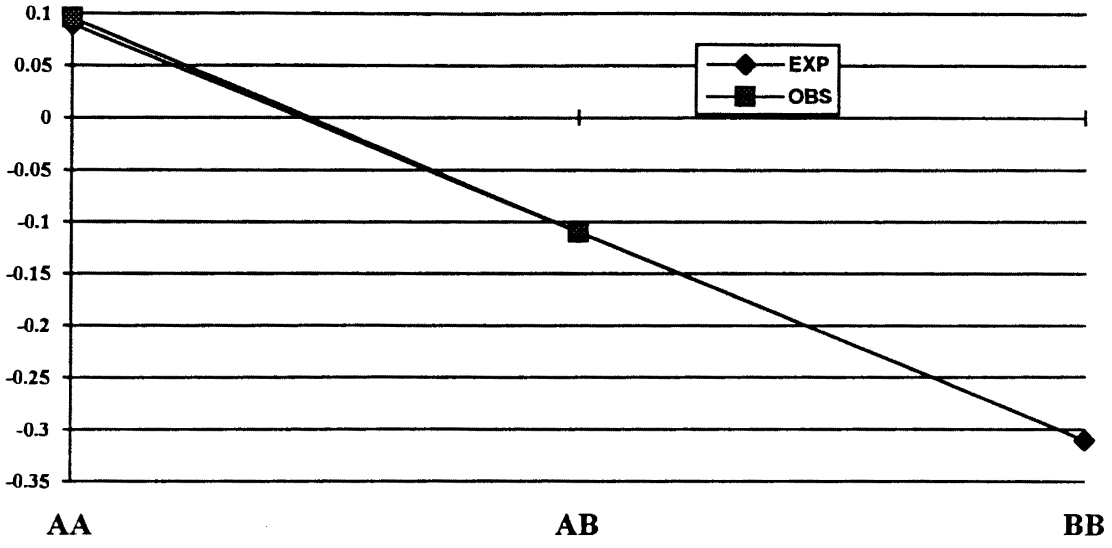
GENE	EPD	P-VALUE	R^2
GH	Marbling	P = .07	.22
GH	Fat Thickness	P = .07	.22
PIT	Dir. Weaning Weight	P = .04	.27
PIT	Yearling Weight	P = .02	.32
H-FABP	Birth Weight	P = .0002	.65
H-FABP	Carcass Weight	P = .08	.20
H-FABP	Marbling	P = .07	.21

Table 19. Expected and Observed Average EPD Values for Significant Traits

GENE-TRAIT EPD	A A		A B		B B	
	EXP	OBS	EXP	OBS	EXP	OBS
GH-MARBLING	.09	.096	-.11	-.11	-.31	------
GH-FAT THICKNESS	-.02	-.02	0	0	.02	------
PIT-WEANING WEIGHT	14.6	16	24.0	23.8	33.4	33.8
PIT-YEARLING WEIGHT	24.0	22	42.5	42.8	61.0	60.5
H-FABP-BIRTH WEIGHT	4.7	4.8	2.3	1.9	-.08	0.7
H-FABP-CARCASS WEIGHT	5.8	4.5	-1.7	.17	-9.2	-24
H-FABP-MARBLING	-.09	-.08	.08	.07	.25	.28

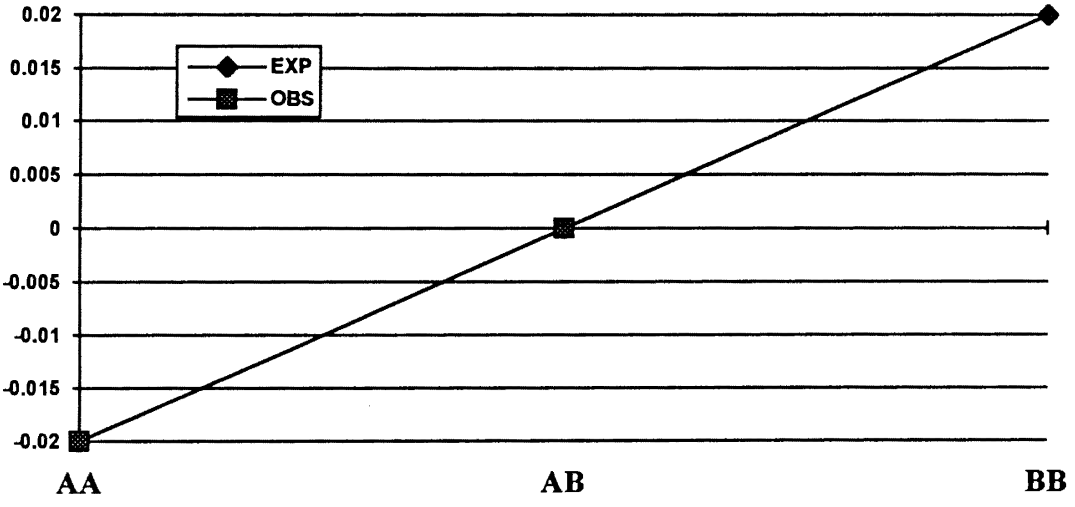
EXP = Expected Average EPD value based on Regression Equation; OBS = Observed Average EPD value based on 16 Angus Sire EPDs. No BB genotype was present for GH gene in the 16 Angus sires evaluated.

Figure 6. Regression of Marbling EPD on Growth Hormone Genotype



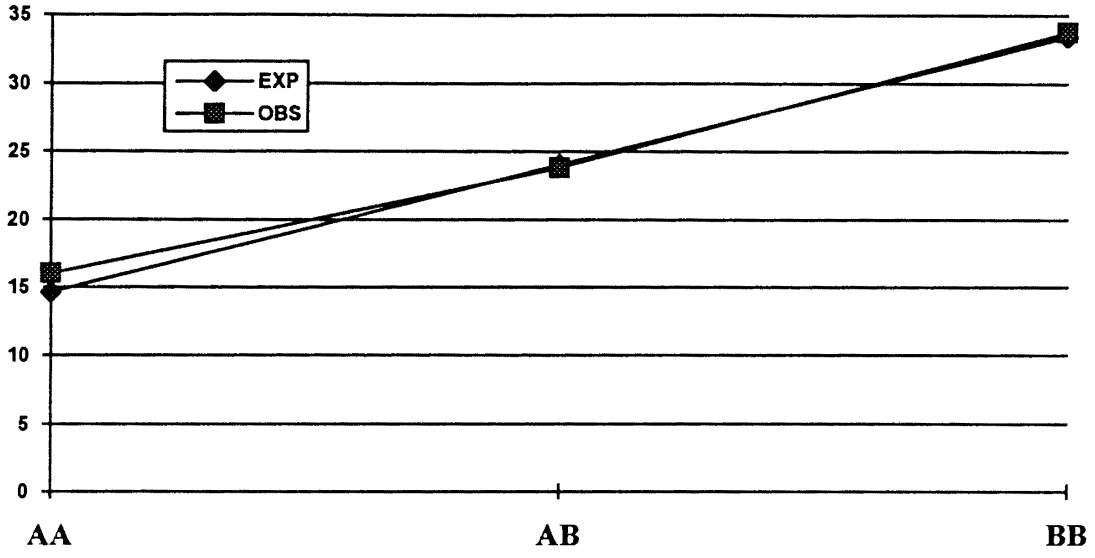
EXP = Expected values based on regression equation; OBS = Actual observed values

Figure 7. Regression of Fat Thickness EPD on Growth Hormone Genotype



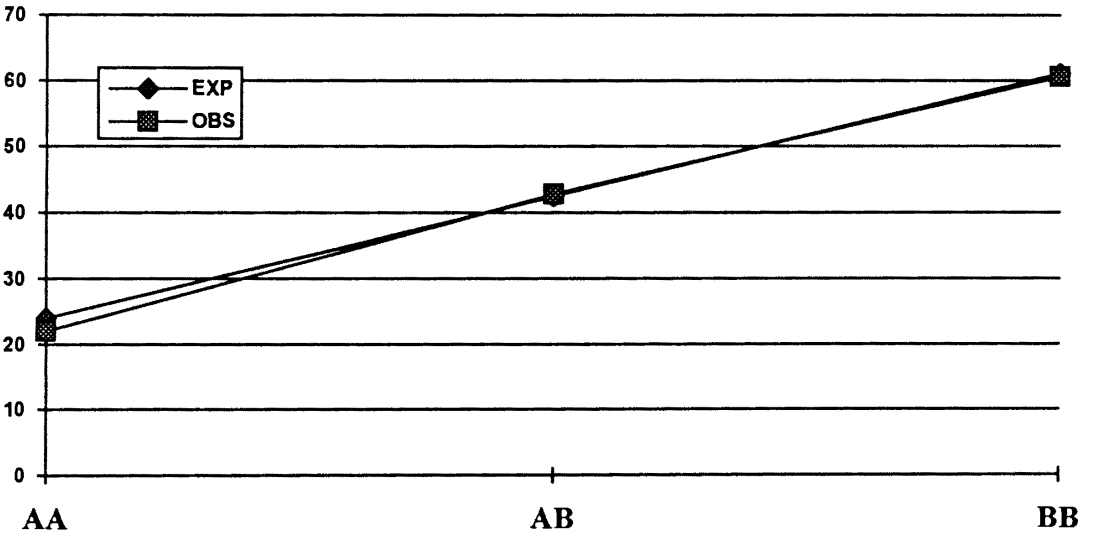
EXP = Expected values based on regression equation; OBS = Actual observed values

Figure 8. Regression of Direct Weaning Weight EPD on Pituitary Transcription Factor-1 Genotype



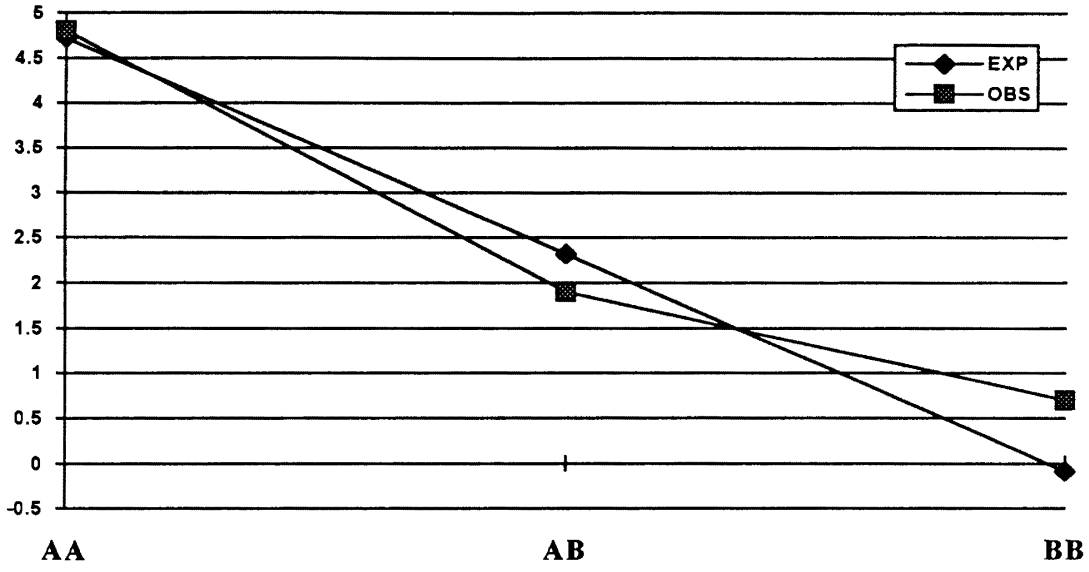
EXP = Expected values based on regression equation; OBS = Actual observed values

Figure 9. Regression of Yearling Weight EPD on Pituitary Transcription Factor-1 Genotype



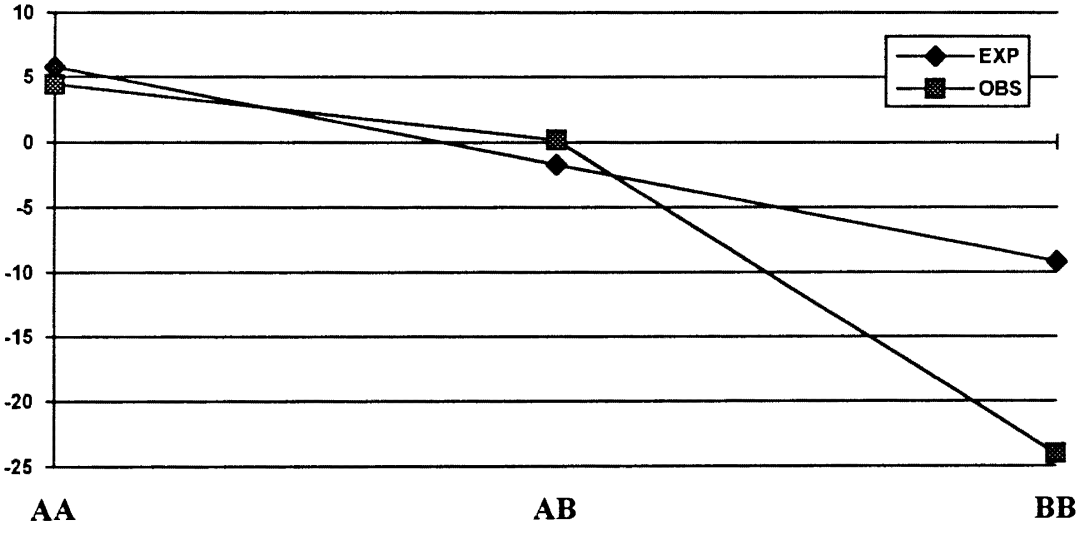
EXP = Expected values based on regression equation; OBS = Actual observed values

Figure 10. Regression of Birth Weight EPD on Heart-Fatty Acid Binding Protein Genotype



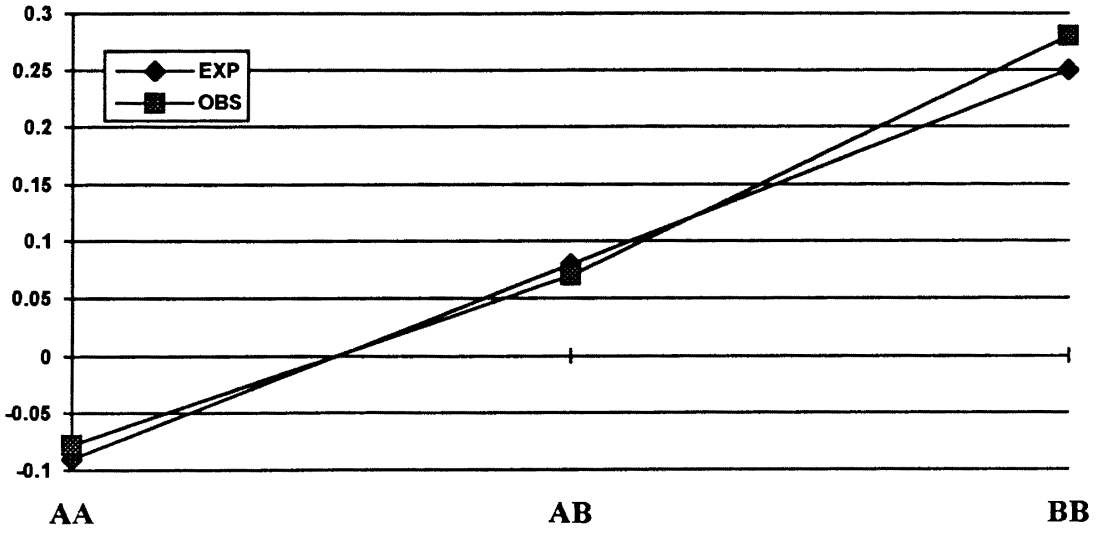
EXP = Expected values based on regression equation; OBS = Actual observed values

Figure 11. Regression of Carcass Weight EPD on Heat-Fatty Acid Binding Protein Genotype



EXP = Expected values based on regression equation; OBS = Actual observed values

Figure 12. Regression of Marbling EPD on Heart-Fatty Acid Binding Protein Genotype



EXP = Expected values based on regression equation; OBS = Actual observed values

CHAPTER V

DISCUSSION

Summarized Review of Results

The GH and B-Lac allele frequencies were significantly different between high and low marbling sire groups. The GH, B-Lac and H-FABP allele frequencies were significantly different between high and low marbling offspring groups. The general effects of K-Cas genotype were on carcass and subprimal weights, the size and weight of the longissimus dorsi muscle and the percentages of fat. The consensus effect of the K-Cas genotype on these carcass traits was the dominant action of the A allele for increased area, weights or percentages on all affected traits. The GH genotype affected carcass and subprimal weights when taking the consensus results from both statistical analyses. The overdominance of the heterozygote was distinguished as the gene action for GH, with the BB genotype intermediate in value between the heterozygote and the AA genotype. GH genotype also had a significant affect on shear force values with the BB genotype having the highest shear force values. The Pit-1 genotype influenced carcass, subprimal and seam fat weights, as well as the percentages of fat, lean and bone. Contrasts for both analyses identified additive gene action with the AA genotype consistently having the highest values for carcass and subprimal weights, as well as seam fat weights. The AA genotype also had the highest percentage fat and the lowest percentage of lean. The dominant gene action of B-Lac was identified in both analyses for RIB weight, with the B allele dominant for decreased RIB weights.

Explanation for Allele Frequency Differences

The differences detected in allele frequencies between high and low marbling offspring groups are representative of allele frequency differences between the high and low marbling sires. The significance of the H-FABP loci being significant in the offspring, but not in the sires, could result from allele frequencies in the dam line (values unknown), or from the larger population size of the offspring groups, which would allow for the detection of a significant difference between high and low marbling groups based on increased statistical power. The fact that the level of significance increased from differences seen in the sire groups to differences detected in the offspring groups could also result from population size differences between offspring and sire numbers or the dam line allele frequencies (values not known). These observed differences between allele frequencies in the high and low marbling sire groups, as well as in the offspring groups could have several explanations.

One possible explanation for significant differences for allele frequencies between the high and low marbling groups is that different phenotypes result from different genotypic compositions. When genes that have different allelic forms are involved in affecting a phenotypic trait which is selected for, and the allelic forms of that gene are responsible for creating variation in the phenotypic trait, then the allelic forms of that gene that produce a desired phenotype are selected, while allelic forms that produce undesirable phenotypes are selected against. Therefore, phenotypic differences can generally be explained to some extent by genotypic differences. The twelve sires used for this project differed in marbling EPD values, and when scrutinizing the list of EPD values presented for the Angus sires in Table 3 and Table 4 the low marbling sires tend on average to have higher growth EPD values. Therefore, the differences seen in allele frequencies between the high and low marbling sire groups may represent the fact that B-Lac and GH loci are

some how affecting the phenotypes which are different between the high and low marbling groups. Since carcass and subprimal weights were significantly affected by both B-Lac and GH, it is possible that the allele frequency differences may reflect the different phenotypic tendencies of the high and low marbling groups. This theory would be supported since the carcass and subprimal weights would be influenced by the body weight of the animal which could be represented by the difference in growth EPDs between the high and low marbling group sires. The significantly different allele frequencies could also be explained due to chance. Since the population for this study was created using only twelve Angus sires, the differences in allele frequencies could be due to sampling error.

Due to the small number of sires used to create this population, the allele frequency differences seen at the B-Lac and GH loci between marbling groups are not very informative. With the selection of only twelve Angus sires, the odds that sampling error could explain the differences in allele frequencies is quite high. The allele frequency differences between high and low marbling groups for the GH locus are the most likely to explain differences in phenotypic traits. This is due to the fact that the polymorphism evaluated at this locus directly affects the polypeptide sequence (Zhang et al., 1992) and could be directly influencing the action of growth hormone. Supporting this conclusion are studies that have shown that beef cattle treated with exogenous growth hormone generally experience an increase in lean tissue accretion and decreased lipid deposits (Moseley et al., 1992; Groenewegen et al., 1990).

The fact that the B-Lac polymorphism affects a milk protein polypeptide sequence (Medrano and Aguilar-Cordova, 1990b) would seem to have little relevance to an animal's body composition. Also, only one significant carcass trait was identified for the B-Lac locus out of the several that were evaluated.

Since the H-FABP polymorphism identified here is located in a DNA sequence with high homology to the heart-fatty acid binding protein sequence we do not know if the

polymorphism has any effect on protein production, or if any protein is produced for the H-FABP sequence. Once again, no carcass traits were identified that were significantly affected by the H-FABP genotype and the allele frequency differences identified may be significant due to dam line influence.

In summary, the allele frequencies presented here provide little more information other than to describe the population and possibly provide indications of which loci may be directly affecting or serving as markers for phenotypic differences between the high and low marbling groups. This interpretation is also clouded by the fact that the HM and LM sire groups may vary in traits other than marbling and growth EPD values.

Allele frequencies for all of the five loci tested were not significantly different from the expected values under Hardy-Weinberg Equilibrium. It is obvious that this population does violate the Hardy-Weinberg conditions due to the fact that the sires were selected, and the population is far from large. The fact that the allele frequencies were not significantly different from Hardy-Weinberg may indicate that the genes evaluated in this study are not largely influencing the phenotypic traits that the sires were selected on, namely marbling EPD. If one of the five evaluated loci were undergoing selection pressure in this Angus-cross population due to favorable effects on a phenotypic trait (such as marbling EPD) then we would expect that locus to violate the law since the population would not be randomly mated with concern to that locus. Another possibility, is the fact that the Hardy-Weinberg law appeared not to be violated due to the small population size which would make small differences seem insignificant, where a larger population with more statistical power may recognize a small difference in genotypic categories to be significant.

Implications of Significant Genotype Effects on Carcass Traits

Several carcass traits were identified as being significantly affected by one or more of the five evaluated markers. This provides an indication that some of these genes could serve as potential markers for carcass traits in beef cattle. The fact that sire was significant for almost every trait except for NTEB and NTEBN could easily be explained by the variation in sire EPD values which should have influenced the traits in their offspring. The fact that sire was not significant for NTEB or NTEBN is not surprising since a difference in bone weight or bone percentage among the sires is unlikely since it was not used as a selection criteria for the sires as was the carcass characteristic of marbling. The explanation for the different actions of NTEB or NTEBN when compared to the other carcass traits may be due to the fact that all other traits are fat or lean tissue values, unlike bone. Growth Hormone expression is determined by the presence of pituitary transcription factor-1 (Ingraham et al., 1990) and the presence of growth hormone influences traits such as tissue and bone growth. Since bone is primarily a mineral deposition site, growth hormone may not influence bone growth in the same manner as the fat or lean tissue growth.

The significant effect of the composite variable for these same traits is probably due to the time of slaughter or sex of the animal. The variation in kill time should have been sufficient to generate differences in all carcass traits based on the animals age at slaughter. The sex of the animal would be expected to generate differences in carcass traits as well. Steers tend to be leaner than heifers, and have heavier slaughter weights and larger REAs. In some situations the interaction between TYS and genotype was significant, however it is difficult to sort out what is the cause of this significant interaction.

Kappa-Casein

The summarized results suggest that the K-Cas polymorphism may serve as a potential marker for several carcass traits including carcass and subprimal weights, lean tissue weights, and the percentage of fat. The K-Cas polymorphism does affect the polypeptide sequence of a milk protein (Medrano and Aguilar-Cordova, 1990a), however, it is unlikely that this polymorphism would have any direct affect on an animal's body composition. Therefore, it is likely that this polymorphism is actually serving as a marker for another gene which is located nearby on bovine chromosome 6 (Barendse et al., 1994). Genes located nearby the K-Cas locus on chromosome 6 include albumin, the gamma-amino butyric acid receptor alpha 2 and the H-FABP marker (Barendse et al., 1994). It seems doubtful that any of these genes may play a role in body composition. However, if the H-FABP marker is a pseudogene for the true heart-fatty acid binding gene, it may mean that the real gene for heart-fatty acid binding protein is nearby on bovine chromosome 6. Therefore, it may be possible that the K-Cas polymorphism is serving as a marker for the heart-fatty acid binding gene. The results attained for the traits significantly affected by K-Cas genotype are encouraging. The RIB weight should be a direct function of HCW, and if REA is significant then one would expect that the NTEEYE weight would also be significant since both are measurements on the longissimus dorsi muscle. The K-Cas genotype significantly affected all of these traits, and the same gene action (dominance) was identified for the same traits in both least-squares and mixed model analysis. Results such as these would provide encouragement for further study of the K-Cas polymorphism on carcass characteristics. However, due to the poor genotypic distribution and the resulting high standard errors within this evaluated population for the K-Cas BB genotype, it is difficult to determine the true mean of the BB homozygote on carcass traits without a large amount of error. When considering the fact that the AA and

AB genotypes tended to be significantly different from the BB genotype, one must consider if the significant effects witnessed here are real, or are simply due to the extreme values of the few BB animals in this evaluation. In order to make sound conclusions about the genotype affect of K-Cas on carcass traits, an analysis must be done with larger numbers of animals in each genotype classification.

Beta-Lactoglobulin

The polymorphism in the B-Lac gene also results in a change in the polypeptide sequence (Medrano and Aguilar-Cordova, 1990b) much the same as that for K-Cas. Therefore, once again it would be doubtful that this polymorphism would be directly influencing body composition. However, it is possible that this polymorphism could serve as a useful marker for other genes located nearby on chromosome 11 (Bishop et al., 1994) which may affect carcass traits. The fact that B-Lac genotype significantly affected RIB weight in both analyses, with similar contrast results is encouraging. However, if this is a real effect of the B-Lac gene, or a gene nearby, we would expect to see other traits such as HCW and/or NTERIB also affected. Not only are other related traits not affected, but the level of significance that the B-Lac genotype affects RIB weight for both analysis is borderline significant. Still another problem arises with the affect of B-Lac on RIB when we consider that the AA genotype group only contains a few animals, and once again the AA genotype is always significantly different from the AB and BB genotypes. The significance of the B-Lac genotype on RIB weight for this study has little validity, more animals with the AA genotype would be needed to improve the confidence of this finding.

Growth Hormone

The GH polymorphism, much the same as the K-Cas and B-Lac polymorphism, does yield a polypeptide change in the growth hormone protein (Zhang et al., 1992). This polymorphism is much more encouraging then the two milk protein polymorphisms due to the effect that growth hormone has on body composition and body metabolism of proteins and lipids (Groenewegen et al., 1990; Press, 1988). Based on the known effects of the growth hormone protein, there is the possibility that associations between this polymorphism and carcass traits may represent a direct effect of the GH polymorphism on body composition. The associations between GH and carcass and subprimal weights provide interesting implications for how the change in the growth hormone protein may be influencing the role of growth hormone. Since the GH polymorphism represents different protein forms, the two forms for growth hormone may have different physiological effects, such as their affinity for growth hormone receptors. If one protein form does have a higher affinity for the receptor, it may in turn affect growth and body composition to a larger degree. The other alternative is that this polymorphism in the GH gene may serve as a marker for another gene located nearby on bovine chromosome 19 (Barendse et al., 1994). The fact that the genotypic distribution for the GH polymorphism is evenly distributed provides support that these associations between GH genotype and carcass traits may be valid. The largest significant differences between GH genotypes are seen between the AA and AB genotypes, which happen to possess a relatively equal number of animals in both genotype categories. The fact that both HCW and RIB weight were found to be significant in both analyses further provides support that these associations between GH genotype and carcass traits may have real implications. The sire regression analysis indicated that GH genotype affected marbling and fat thickness EPDs. These results, though not supported by the least-squares and mixed model analyses, would seem logical

based on the suggested effects that growth hormone has on body composition (Groenewegen et al., 1990). However, the few animals used for the sire regression analysis provide little power and validity to this analysis.

Pituitary Transcription Factor-1

The polymorphism located in the Pit-1 gene, unlike the others discussed so far, does not directly affect the polypeptide sequence. This Pit-1 polymorphism is located in an intron region (Moody and Pomp, 1994). Therefore, the significant associations detected between this polymorphism and carcass traits could serve as an indication that the Pit-1 gene or a DNA region located nearby on bovine chromosome 1 (Barendse et al., 1994) is having an effect on the carcass traits. The idea that the Pit-1 gene may influence carcass traits is indicated by the fact that the Pit-1 protein is necessary for the proper transcription of the growth hormone gene (Ingraham et al., 1990; Karin et al., 1990). Based on the impact that GH has on body composition and body metabolism (Groenewegen et al., 1990; Press, 1988), the factors involved in controlling the expression of the GH gene would also be important in the regulation of the effects of growth hormone. The fact that Pit-1 had consistent effects on HCW, RIB and NTERIB for both statistical analyses supports the idea that the Pit-1 genotype may have a real impact on these carcass traits. The results from the sire regression analysis indicate that the Pit-1 genotype is affecting yearling and direct weaning weight EPDs. This would seem to support the idea that if Pit-1 influences growth, we would expect heavier carcass and subprimal weights. Unfortunately, the AA genotype which has the heaviest carcass and subprimal weights has the lowest growth EPDs. The small number of animals used for the sire regression analysis should be enough to discredit the results found when it is in complete disagreement with the offspring carcass results. The effect of Pit-1 genotype on

NTESM, and not on the other carcass fat values such as MARB and NTESC may provide support for the idea that different adipose tissue deposits are influenced by different controlling factors. The most discouraging factor about the results for the Pit-1 genotypes may be that the AA homozygote was always the genotype classification that yielded a significant effect of the Pit-1 genotype. The AA genotype classification has the lowest number of animals and therefore, the largest standard error values. This information would tend to discredit the results obtained in this study.

Heart Fatty Acid Binding Protein Marker

The H-FABP genotype significantly affected AWW means. Combined with these results, the sire regression analysis indicated that the H-FABP genotype significantly affected birth and carcass weights, as well as marbling EPDs. Due to the fact that this H-FABP marker is not a known DNA sequence that produces a functional protein, the conclusion must be that the H-FABP genotype would serve as a marker for other traits on bovine chromosome 6 (Appendix M). However, the idea of H-FABP even serving as a marker is slim based on the low number of animals in the AB genotype, and the complete lack of the BB genotype classification. The results from the sire regression were completely unfounded according to the least-squares and mixed model analysis. Further investigation of the functionality of H-FABP as a marker for carcass and production traits is warranted, however, the significance of its associations detected in this study are most likely due to chance.

Limitations of this Study

When using a level of significance at P<.10 we would expect to find 11.5 (23 traits x 5 markers x .10) significant associations between markers and phenotypic traits. If the significance level is lowered to P<.05 we would expect to find approximately 6 (23 traits x 5 markers x .05) significant associations between markers and traits. Therefore, the large number of associations identified between evaluated loci and carcass traits in this study could be largely due to chance. This high probability of identifying associations between markers and phenotypic traits by chance should cause caution when evaluating the results presented in this thesis. This reason requires that associations identified here between markers and traits be confirmed through future research before conclusions are made.

Another major limitation of this DNA marker analysis study is the small number of animals in some genotypic classes. The half-sib family utilized was created using sires selected based on marbling EPD. Therefore, there was no control over sire genotype for the markers evaluated. In DNA marker analysis, only half-sib families with a heterozygous common parent are of interest (Soller and Genizi, 1978). In the majority of the markers evaluated in this study, the genotypes for the twelve Angus sires were predominantly one of the two homozygous genotypes. Due to this allele frequency bias in the sires, the offspring population had certain genotypic classifications with few, and in some cases, no representative animals. These genotypic classifications then become very susceptible to having their mean values for the evaluated carcass traits heavily influenced by the extreme phenotypes of animals in that genotype classification. Therefore, due to the small number of offspring in some of the genotype classes which resulted from the lack of heterozygous sires used to create the population, there is a definite limitation of this population in its usefulness of detecting markers associated with QTL.

Another striking limitation of the population used in this study is the lack of statistical power due to the low number of animals. The number of animals needed to detect significant associations between marker loci and QTL has been estimated in the thousands depending on several variables such as heritability and allele frequencies (Soller and Genizi, 1978; Soller et al., 1976; Soller, 1978). The small number of animals utilized for this study can not provide definitive results about how the markers evaluated affect carcass traits. The most that can be gained from this study is the possible associations between these markers and carcass traits.

The cost of creating livestock populations that are large enough, and that have enough heterozygosity to allow for the testing of several markers at once in one population is extreme. The utilization of the daughter and granddaughter designs (Weller et al., 1990) in beef cattle populations may serve as a useful tool to decrease the cost in the search for markers linked to QTL. The ideal population for the study of DNA markers associated with QTL would involve a large population created by the cross of two inbred lines with varying phenotypic traits. The resulting heterozygous F1 would then be intermated to produce the F2. The F2 would serve as a useful population for DNA marker study since it would have fairly equal distributions of genotypes and a wide range in phenotypic values.

The Angus-cross population used for this DNA marker study was created to produce offspring that could be studied for production, reproductive and meat science purposes. This population also offered the opportunity for the evaluation of DNA markers since there were half-sib families with large amounts of variation for carcass traits. At the time that this research project was begun, the bovine genome maps (Barendse et al., 1994; Bishop et al., 1994) were still not in existence. Therefore, the candidate gene approach was used. The candidate gene approach is simply the selection of certain genes to target as having possible effects on the phenotypic traits of interest. If

this project was started over today, the markers published in the bovine genome maps would be screened for effects on carcass traits.

Conclusions

The associations of several of the evaluated loci with carcass traits in this study should be tempered with the knowledge of the low statistical power and the population design. Continued investigation of the roles that K-Cas, GH and Pit-1 genotypes have on body composition are needed. The percentage of total and residual variation explained by genotype for several of the carcass traits (Table 13) would suggest that if the associations between genotypes and carcass traits seen here are real, that these markers could be utilized in marker assisted selection. These results provide a foundation for future research with larger population sizes and more genetic markers. A larger phenotypically diverse F2 population established with more even distributions for each genotype where alleles can be traced from parent to offspring would serve as a useful population for further carcass studies. However, the production of beef cattle populations that fit this description are costly and take long time periods to create.

The use of genetic markers in combination with phenotypic data could have large influences on increasing the accuracy of selection, reducing the generation interval and increasing selection differentials (Soller and Beckmann, 1983; Kashi et al., 1990; Meuwissen and van Arendonk, 1992) without the costly progeny and sib testing used today . If further research confirms the findings indicated here, the genes for K-Cas, GH and Pit-1 may eventually serve as markers to provide additional information for the prediction of growth and carcass EPDs.

To conclude, the objectives of this study were attained. The identification of markers associated with carcass traits in beef cattle was accomplished. The validity of

these markers is still questionable, however, with the existing population used for this research the results are as realistic as possible without further investigation. New DNA markers for the bovine genome were identified in LPL and the H-FABP marker. Both of these markers have been mapped to their respective chromosomal location in the bovine genome. The continued search for DNA markers associated with carcass traits, along with the further investigation of the effects of the markers discussed in this paper, could potentially make marker assisted selection a reality. The use of MAS could help to accomplish the beef industries goal of a fast growing market animal with a high percentage of lean, a low percentage of fat and a desired palatability level.

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APPENDIX A --NEBRASKA PRODUCTION STUDY

The results and details for the study, using the same population as described in this research, for the evaluation of production traits can be found in Vieselmeyer et al. (1994). An abstract summarizing these results is also published (Vieselmeyer et al., 1993).

Results of this study found that calving weight or degree of dystocia were not influenced by sire group. However, marbling group did affect weaning weights for both steers and heifers. The actual weaning weights for steers and heifers sired by low marbling sires were 26 and 34 pounds heavier, respectively, than steers and heifers sired by high marbling bulls. When actual weaning weights were adjusted to 205-day weaning weights based on the Beef Improvement Federation guidelines, the difference was still present. The steer and heifers sired by low marbling bulls weighed 16 and 23 pounds heavier than steers and heifers sired by high marbling bulls, respectively, after adjustment. The weight difference between the low and high marbling group steers at slaughter was less than the weight difference witnessed at weaning and at the onset of the finishing period. The weight difference between high and low treatment heifers did not change.

Over 70% of offspring carcasses from sires with high EPDs for marbling graded U.S. Choice versus less than 50% from the low EPD sires. The depth of subcutaneous fat measured at the 12th rib was not different for either sex or marbling group across both slaughter times. The percentage of animals from the high and low marbling treatments within each U.S.D.A. yield grade was similar.

The weight differences seen at weaning were a reflection of the weaning weight EPDs of the sires. The low marbling bulls had higher growth EPD's due to sampling error. Bull selection was based primarily on marbling EPDs. This study indicates that Angus sires can be selected to produce progeny that have an increased ability to grade Choice without increasing yield grade.

APPENDIX B--NEBRASKA CARCASS STUDY

The results and details for the study, using the same population as described in this research, for the evaluation of carcass and palatability traits can be found in Gwatney et al. (1994). An abstract summarizing these results is also published (Gwartney et al., 1993).

The high marbling steers consistently had more marbling than the low marbling steers at any set time. The rate that marbling was deposited for the steers was similar between the high and low marbling EPD treatments. The marbling score for the high marbling EPD heifers increased at a faster rate than the low marbling heifers as fat depth increased over time. Therefore, high marbling steer or heifer carcasses with a set amount of 12th rib fat would consistently have higher marbling scores than low marbling steer or heifer carcasses at the same 12th rib fat depth. High marbling heifer carcasses had less 12th rib fat and less estimated kidney, pelvic and heart fat as a percentage of hot carcass weight than the low marbling heifers. Heifer carcasses from the low marbling EPD group had heavier hot carcass weights and larger ribeyes. After adjustment for marbling score, no differences were identified for any steer carcass traits between the high and low marbling EPD groups. The heifers from the high marbling group had less fat as a percentage of the wholesale rib weight than those from the low marbling EPD group for all depots (except marbling). No differences were detected in steer rib fat percentages between marbling EPD groups. Steers in the high marbling group had less subcutaneous fat as a percentage of quarter weight in the forequarter and hindquarter sections. Also, high marbling EPD group steers had less total fat expressed as a percentage of hindquarter weight. Seam fat was not affected by marbling EPD treatment for either sex. Neither a trained panel evaluation or shear force analysis using the Warner-Bratzler shear found any differences in tenderness, for steers or heifers. No differences were observed in juiciness, flavor or connective tissue.

These results indicate that it is possible to maintain marbling score while decreasing fat deposition in other depots of the carcass through the use of carcass EPDs.

APPENDIX C--NEBRASKA REPRODUCTIVE STUDY

The results and details for the study, using the same population as described in this research, for the evaluation of reproductive traits in heifers can be found in Bergfeld et al. (1993). Heifers produced from the mating of 12 Angus sires (6 high marbling EPD, 6 low marbling EPD) to MARC II composite cows (1/4 Hereford, 1/4 Angus, 1/4 Simmental, 1/4 Gelbvieh) were produced during 1990 and 1991. These heifers were used to test for any difference in age of puberty between heifers sired by high or low marbling EPD bulls. Puberty was determined via blood progesterone concentrations.

No differences in age at puberty was detected for heifers between treatment groups. Heifers bred for high marbling did not reach an earlier physiological maturity, as measured by age at puberty, than heifers bred for low marbling. The heifers born in 1990 tended to have heavier weaning weights than heifers born in 1991. The mean yearling weights for both high and low marbling groups did not differ significantly in 1992. However, the mean yearling weight was an average of 45 pounds heavier in 1992 than the mean in 1991. This study did not find any significant correlation between age at puberty and yearling weight although all heifers were fed the same ration and projected to gain 1.1 to 1.4 pounds per head daily.

In summary, heifers sired by high marbling bulls did not differ significantly in age of puberty compared to heifers sired by low marbling bulls.

APPENDIX D--ANGUS ASSOCIATION MARBLING SCALE

QUALITY GRADE	MARBLING	NUMERICAL SCORE
PRIME^{+}	ABUNDANT	10.0 - 10.9
PRIME°	MOD. ABUNDANT	9.0 - 9.9
PRIME^{-}	SL. ABUNDANT	8.0 - 8.9
CHOICE^{+}	MODERATE	7.0 - 7.9
CHOICE°	MODEST	6.0 - 6.9
CHOICE^{-}	SMALL	5.0 -5.9
SELECT	SLIGHT	4.0 - 4.9
STANDARD	TRACES	3.0 - 3.9
STANDARD	PRAC. DEVOID	2.0 - 2.9
UTILITY	DEVOID	1.0 - 1.9

APPENDIX E--DNA EXTRACTION FROM BULL SEMEN

Note: Wear latex gloves throughout procedure.

1) Empty entire straw into 2.0 ml microcentrifuge tube (apprx. 0.5 ml) or put 0.5 ml of semen into 2.0 ml microcentrifuge tube. Add 1 ml ddH_20, mix briefly, then spin at 8000 RPM for 5 min to pellet sperm.

2) Carefully pour or aspirate off supernatant being careful not to disturb the pellet. If floating milky substance sticks to the inside of the tube, remove with a sterile cotton swab being careful not to touch pellet with swab.

3) Resuspend pellet in 750 µl STES digestion buffer (Appendix I) and mix well.

4) Add 15 µl Proteinase K (AMRESCO Inc., Solon, OH) (20 mg/ml) and 40 µl DTT (1 M in 10 mM NaAc) (Fisher Scientific, Fair Lawn, NJ) and mix by rocking back and forth. Incubate overnight at 37° C or incubate for 2 hours at 57° C (at 1 hour mix tube thoroughly).

5) Add 750 µl of Tris buffered phenol (AMRESCO Inc., Solon, OH) then put on rotator or mix back and forth by hand 5 mins (be sure tubes caps are tightly secured).

6) Centrifuge for 5 mins at 3000 RPM.

7) Aspirate off aqueous (top) phase into labeled 2 ml tube. Add 1X TE (Appendix I) solution (in 50 µl increments) to restore total volume to 750 µl. Add 750 µl Tris buffered phenol/chloroform:isoamyl alcohol (AMRESCO Inc., Solon, OH) and mix by rocking for 5 mins.

8) Centrifuge for 5 mins at 3000 RPM.

9) Aspirate off aqueous phase into labeled 2 ml tube, add an equal volume of chloroform:isoamyl alcohol (Fisher Scientific, Fair Lawn, NJ) and rock for 5 mins.

10) Centrifuge for 5 mins at 3000 RPM.

11) Aspirate aqueous phase into labeled 2 ml tube.

12) Add 1/10 volume 3 M sodium acetate (NaAc) (Fisher Scientific, Fair Lawn, NJ), cap tubes and mix well by rocking back and forth.

13) Add 2 volumes of -20° C 100% EtOH (AAPER Alcohol and Chemical Co., Shelbyville, KY) then rock and roll gently until "angel hair" precipitate forms. Spool out precipitate with curved tip pasteur pipette, wash with 70% EtOH, place rod in clean, labeled 1.5 ml tube and place in vacuum over 2-12 hours to dry. If no "angel hair" is visible, place tube in -80° C one hour then centrifuge at 10,000 RPM for 15 min at 4° C. Pour or aspirate off supernatant and dry pellet in vacuum oven or Speed-Vac.

14) If DNA precipitate was visible in the previous step without centrifugation, then resuspend in 200 µl 1X TE. If centrifugation was necessary then resuspend in 50 µl 1X TE.

15) Read absorbance on spectrophotometer at 260 & 280 nm, calculate concentration and 260/280 ratio then make PCR working solution at 50 ng/µl.

APPENDIX F--DNA SALT EXTRACTION PROTOCOL

Note: Wear latex gloves throughout procedure.

1) Spin tubes of blood in centrifuge, 2000 RPM for 10 mins at 4° C, acceleration of 8, deceleration of 8.

2) Aspirate off most of serum and discard, then carefully aspirate off buffy coat and any red blood cells that come with and put in a 15 ml centrifuge tube. Add prewarmed red blood cell lysis buffer (Appendix I) to bring volume to 14.5 ml. Incubate 10 mins @ 37° C.

3) Spin to pellet white blood cells. Spin at 2000 RPM, for 10 mins at 4° C, acceleration of 8, deceleration of 8.

4) Pour off supernatant and briefly blot tube upside down on kimwipe. Resuspend pellet in 5 ml of 0.15 M NaCl (Sigma Chemical Company, St. Louis, MO) wash lid with sterile 0.15 M NaCl in squirt bottle before screwing on lid. Spin tubes at 2000 RPM, for 10 mins at 4° C, acceleration of 8, deceleration of 8.

5) Repeat step 4 if needed to remove all red blood cell remnants. Resuspend the cleaned pellet in 4 ml of Nuclei Lysis Buffer (Appendix I), add 300 µl of 10% SDS (United States Biochemical Corporation, Cleveland, OH) and 50 µl of Proteinase K (AMRESCO Inc., Solon, OH) (20 mg/ml). Incubate samples for 1-3 hours @ 57° C.

6) Let tubes cool for 5 mins, then add 1.3 ml of 6 M NaCl (Sigma Chemical Company, St. Louis, MO) and shake vigorously for 15 seconds. Let tubes stand at room temperature for 5 min. Spin tubes at 2700 RPM for 10 mins at 4° C, acceleration of 8, deceleration of 8.

7) Draw off supernatant into another 15 ml tube, add 1 volume iso-propanol (Fisher Scientific, Fair Lawn, NJ), rock and roll until 'angel hair' precipitate forms, then spool out with a glass pipette with a curved tip and dry in vacuum oven.

8) Resuspend in 500 µl 1X TE (Appendix I) and proceed to determine quality of DNA through use of the spectrophotometer machine.

APPENDIX G--DNA EXTRACTION FROM MUSCLE TISSUE

Note: Wear latex gloves throughout procedure.

1) Homogenize approx. 0.5 - 1 gram of lyophilized tissue in 3 ml of Nuclei Lysis buffer (Appendix I). Place the homogenized sample in a 15 ml tube. Rinse the homogenizer with 1 ml of Nuclei Lysis buffer, and add this to the 15 ml tube. Store finished samples on ice until all samples are homogenized.

2) Add 300 µl of 10% SDS (United States Biochemical Corporation, Cleveland, OH) to each tube, and then mix well by shaking. If the sample appears to get thick and non-liquid, 1 ml of Nuclei Lysis buffer can be added. Shake the tube vigorously.

3) Add 50 µl of 20 mg/ml Proteinase K (AMRESCO Inc., Solon, OH) to each tube, mix well.

4) Digest the samples in a shaking incubator at 56° C for a minimum of 3 hours.

5) Let samples sit at room temperature for 5 min. Add 1.3 ml of 6 M saturated NaCl (Appendix I) (Sigma Chemical Company, St. Louis, MO) solution. Shake each tube vigorously for 15 seconds. Let tubes sit at room temperature for 5 min.

6) Spin the tubes at 2700 RPM at 4° C for 10 min.

7) Draw off the aqueous layer, and place in a clean 15 ml tube. Discard the 15 ml tube containing the protein pellet. Add 1 volume of iso-propanol (Fisher Scientific, Fair Lawn, NJ) to the 15 ml tube, and rock and roll gently until DNA precipitates.

8) Spool out DNA with a glass pipette with a curved end, and rinse with 70% ethanol (AAPER Alcohol and Chemical Co., Shelbyville, KY), and place in a clean 1.5 ml tube. Dry on counter overnight (covered), or in vacuum oven. Resuspend in 400 µl of 1X TE (Appendix I).

9) If no DNA precipitates, store the 15 ml tube in a -80° C freezer for at least one hour.

10) Transfer these samples to appropriate centrifuge tubes and spin at 15,000 RPM for 15 min at 4° C. You should get a white DNA pellet in the bottom of your tube. Evaporate the iso-propanol (Fisher Scientific, Fair Lawn, NJ) and resuspend the pellet in 400 µl 1X TE.

* If samples do not PCR or will not resuspend well, then it may be necessary to take the samples through a short phenol-chloroform clean-up protocol (Appendix H).

APPENDIX H--PHENOL CHLOROFORM CLEAN-UP

Note: Wear latex gloves throughout procedure.

1) Take the respective sample and resuspend it in 400 µl of 1X TE (Appendix I) the best that you can.

2) Transfer the sample to a 2.0 ml tube.

3) Add 1 ml of Phenol (AMRESCO Inc., Solon, OH). Mix well by rocking back and forth.

4) Centrifuge at 3000 RPM for 10 min at 4° C.

5) Draw off supernatant from the tube and place in a clean 2.0 ml tube.

6) Repeat steps 3-5, except use Phenol/Chloroform (AMRESCO, Inc., Solon, OH).

7) Repeat steps 3-5, except use Chloroform (Fisher Scientific, Fair Lawn, NJ).

8) After adding the Chloroform and spinning the samples, draw off the supernatant and place in a clean 2.0 ml tube.

9) Add 1/10 volume of 3 M NaAc (Fisher Scientific, Fair Lawn, NJ).

10) Add 2 volumes of ice cold 100% EtOH (AAPER Alcohol and Chemical Co., Shelbyville, KY). Rock tube gently and DNA should precipitate.

11) Remove DNA with glass pipette with curved end, and rinse with 70% ethanol. Place in a 1.5 ml tube. Dry, and resuspend in 200-400 µl of 1X TE (Appendix I).

APPENDIX I--SOLUTIONS FOR DNA WORK

1 Liter of 1X TE
1.576 g Tris-HCl, pH 8.0
0.37224 g EDTA
Fill to 1 Liter with ddH20

6 M NaCl
350.64 g NaCl
Dissolve in ddH20 with heat
Bring to 1 Liter with ddH20

Nuclei Lysis Buffer
0.01 M Tris-HCl, pH 8.0
0.4 M NaCl
0.002 M EDTA, pH 8.0

Red Blood Cell Lysis Buffer
0.017 M Tris-HCl, pH 8.0
0.14 M NH_4Cl

STES Digestion Buffer
10 mM Tris, pH 8.0
10 mM EDTA
50 mM NaCl
2.0% SDS

1X TBE
0.09 M Tris-borate
0.002 M EDTA

1% Agarose Gels
100 ml 1X TBE
1 g BioRad Agarose

3% Agarose Gels
100 ml 1X TBE
1.5 g BioRad Agarose
1.5 g NuSieve Agarose

Tris, Tris-Borate, EDTA: Fisher Scientific, Fair Lawn, NJ
NaCl, NH_4Cl: Sigma Chemical Company, St. Louis, MO
SDS: United States Biochemical Corporation, Cleveland, OH
BioRad Agarose: BioRad Laboratories, Hercules, CA
NuSieve Agarose: Midwest Scientific, St. Louis, MO

APPENDIX J--DNA SPECTROPHOTOMETER PROTOCOL

Note: Wear Latex gloves throughout procedure.

1) Turn the spectrophotometer on and allow adequate time for the machine to warm up. This takes approximately 15 minutes when using the Lambda 3B spectrophotometer.

2) Select the size (volume) cuvette needed. Make a blank by putting in the following volumes of ddH20. The regular size needs 2.0 ml solution, the mini 1.0 ml, and the micro 100 µl. Generally a mini cuvette is selected and a 100:1 dilution is made of the sample (10 µl of DNA sample and 990 µl ddH20).

3) Place the blank cuvette (mini cuvette with 1.0 ml of ddH20) into spectrophotometer, and have the machine go to 260 nm wavelength UV light. Once the machine reaches 260 nm then auto zero the machine so the display reads 0.0000.

4) Now prepare your sample cuvette (10 µl DNA sample and 990 µl ddH20). Mix the sample well with a wide tip pipette being sure not to introduce air bubbles into the solution. Insert the cuvette into the spectrophotometer and close the lid. Record the absorbance reading at the 260 nm UV wavelength. If the absorbance reading does not stabalize and is fluctuating widely then your sample may need to be remixed.

5) After recording your 260 nm absorbance reading, then change the UV wavelength to 280 nm. Record the sample absorbance reading at the 280 nm UV wavelength.

6) This DNA sample can now be discarded, and the cuvette should be washed out using the cuvette washer. Rinse the cuvette twice with ddH20 and once with acetone (Fisher Scientific, Fair Lawn, NJ). Continue steps 1-6 until all DNA samples have a 260 and 280 nm absorbance reading. The spectrophotometer should be auto zeroed using a blank cuvette every 5 to 10 samples.

7) Calculations can now be made. The first calculation is to determine the Abs260/Abs280 ratio which gives an idea of the degree of impurities left behind from the extraction procedure. A ratio of 1.6-1.9 is considered satisfactory. A lower ratio implies protein impurity and the higher ratio implies either RNA or solvent impurity.

8) The DNA samples concentration is determined using the following formula:

Abs260 X Dilution factor X 50 ng/ul = DNA concentration in ng/ul

The 50 ng/ul is a given value for mammalian genomic DNA concentration per O.D. unit.

9) Now the working solution can be made. Generally 200 µl of a 50 ng/ul working solution is made. This figures out that your working solution should have a final concentration of 10,000 ng of DNA.

10,000 ng ÷ [conc.] of DNA sample in ng/ul= # ul of DNA sample to add
200 ul - # ul of DNA sample to add = # ul 1X TE buffer to add

10) Mix working solution well and store at 4° C, then store stock concentration at -20°C.

APPENDIX K--PRIMER SEQUENCE TABLE

GENE		PRIMERS (5' TO 3')
K-CAS	**5'-primer**	ATCATTTATGGCCATTCCA
K-CAS	**3'-primer**	AGACAATGTCTCTTCCGCTT
B-LAC	**5'-primer**	TGTGCTGGACACCGACTACAAAAAG
B-LAC	**3'-primer**	GCTCCCGGTATATGACCACCCTCT
GH	**5'-primer**	CCGTGTCTATGAGAAGC
GH	**3'-primer**	GTTCTTGAGCAGCGCGT
PIT-1	**5'-primer**	CAATGAGAAAGTTGGTGC
PIT-1	**3'-primer**	TCTGCATTCGAGATGCTC
LPL	**5'-primer**	TAACTATCCCCTGGGCAATGTGC
LPL	**3'-primer**	TGCAATCACACGGAGAGCTTCC
H-FABP	**5'-primer**	TACCTGGAAGTTAGTGGGACAGC
H-FABP	**3'-primer**	CTTGGCTCTGCTTTATTGACCT

APPENDIX L--BOVINE LPL POLYMORPHISM

Polymorphism. Two alleles of the bovine lipoprotein lipase (LPL) gene were detected from restriction fragment length polymorphism (RFLP) analysis of a Polymerase Chain Reaction (PCR) product digested with *Sau* 96 I (New England Biolabs, Inc., Beverly, MA).

Primer Source. Primers were designed based on bovine cDNA sequence (Senda et al., 1987) to amplify a region corresponding to exon IV, intron IV and exon V of the human LPL gene (Oka et al., 1990). Expected size of the PCR fragment based on human sequence was ~ 925 bp.

Primer Sequence. 5' Primer: 5'-TAACTATCCCCTGGGCAATGTGC-3'; 3' Primer: 5'-TGCAAT CACACGGAGAGCTTCC-3'.

Method of Detection. PCR conditions were: 2.25 mM MgCl, 100 μM of each dNTP (Boehringer-Mannheim, Indianapolis, IN), 0.1 μM of each primer, 50 - 100 ng of genomic DNA and 0.42 U of *Taq* DNA polymerase (Boehringer-Mannheim, Indianapolis, IN) in a final volume of 12 μl. The first cycle of the PCR was 2 min at 95°C, 1 min at 65°C and 2 min at 72°C. This was followed by 29 cycles under the conditions of 1 min at 94°C, 1 min at 65°C and 1 min at 72°C. The PCR ended with a 9 min extension phase at 72°C. PCR product (10.7 μl) for each sample was digested with 1.25 Units of *Sau 96 I* (New England Biolabs Inc., Beverly, MA) at 37°C for 5 hours. Digestion products were electrophoresed in a 3% agarose gel for 1 hour at 73 volts. The agarose gel was stained for 30 min in ethidium bromide (Sigma Chemical Company, St. Louis, MO) (5 μg/ml) and destained for 30 min in ddH_20.

Description of Polymorphism. The PCR product was ~1345 bp (See attached Figure). Digestion with *Sau 96 I* revealed two alleles (See attached Figure). The A allele is cut at two recognition sites, yielding fragments of approximately 700, 435 and 210 bp.

The B allele is cut at three recognition sites, yielding fragments of approximately 495, 435, 210 and 205 bp.

Mendelian Inheritance. Departure from Mendelian inheritance was not detected in 6 full-sib families where 70 animals were evaluated. All animals evaluated had at least one heterozygous parent.

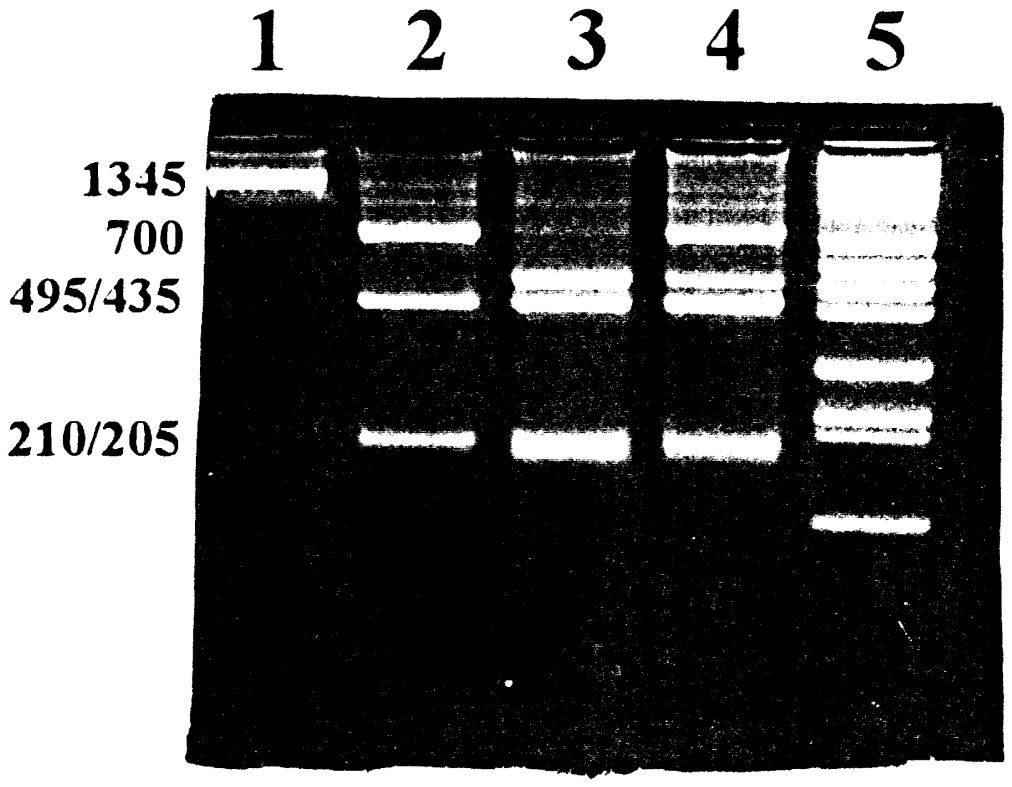
Allele Frequencies. One hundred and two *Bos Taurus* animals were genotyped including Holstein (n=17), Gelbvieh (n=17), Angus (n=24), Hereford (n=23) and Polled Hereford (n=21) breeds. All *Bos Taurus* animals possessed only the A allele. Four Brahman (*Bos Indicus*) animals were genotyped yielding allele frequencies of .875 for the A allele and .125 for the B allele. The B allele has only been identified in *Bos Indicus* and *Bos Indicus X Bos Taurus* crosses. The parents of the International Bovine Reference Family Panel (IBRFP), which included animals of both *Bos Taurus* and *Bos Indicus* descent, had allele frequencies of .94 for the A allele and .06 for the B allele.

Chromosomal Location. Linkage analysis utilizing the IBRFP and the Cattle Genotypic Database determined that the LPL gene is on bovine chromosome 8. This chromosomal assignment confirms an earlier report (Threadgill and Womack, 1991) utilizing physical mapping methods.

Comments. The LPL enzyme is involved with removal of triacylglycerides from the bloodstream through the breakdown of chylomicrons and other lipoproteins (Shore et al., 1959), and is necessary for proper lipid utilization. The LPL PCR product described here contains two of the amino acid codons that make up the catalytic triad, which is believed to be responsible for providing function of the LPL enzyme (Ma et al. 1992). This polymorphism in the bovine LPL gene could serve as a useful marker in the search for quantitative trait loci in resource families with *Bos Indicus* ancestry. The conditions used to amplify bovine LPL produced a PCR product of the expected size in ovine, but no product was obtained in porcine or murine.

Figure Legend

FIGURE 1. Genotypes for LPL on a 4% agarose gel. Lane 1: Uncut PCR product (~ 1345 bp); Lane 2: AA Genotype (~ 700, 435 and 210 bp); Lane 3: BB Genotype (~ 495, 435, 210 and 205 bp); Lane 4: AB Genotype (~ 700, 495, 435, 210 and 205 bp); Lane 5: Boehringer-Mannheim (Indianapolis, IN) Marker VI. The smear of DNA at the top is unseparated bands of 2176, 1766, 1230, and 1033 bp. The remaining bands are 653, 517, 453, 394, 298, 234, 220 and 154 bp in descending order.



APPENDIX M--BOVINE H-FABP POLYMORPHISM

Polymorphism. Two alleles of a DNA region with high homology to the bovine heart fatty acid binding protein (H-FABP) gene were detected through restriction fragment length polymorphism (RFLP) analysis of Polymerase Chain Reaction (PCR) products digested with the restriction enzyme *Rsa I* (GibcoBRL, Grand Island, NY).

Primer Source. Primers were designed based on the bovine heart cDNA sequence for fatty acid binding protein (Billich et al., 1988) to amplify from nucleotide number 66 of the cDNA to nucleotide number 676.

Primer Sequences. 5' Primer: 5'-TACCTGGAAGTTAGTGGACAGC-3'; 3' Primer: 5'-CTTGGC TCTGCTTTATTGACCT-3'.

Method of Detection. PCR conditions were: 0.75 mM MgCl, 100 μM of each dNTP (Boehringer-Mannheim, Indianapolis, IN), 0.1 μM of each primer, 0.42 Units of *Taq* DNA polymerase (Boehringer-Mannheim, Indianapolis, IN) and 50 - 100 ng of genomic DNA in a total volume of 12 μl. The first cycle of the PCR was 2 min at 95°C, 1 min at 55°C and 2 min at 72°C. This was followed by 34 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C. The PCR ended with a 9 min extension phase at 72°C. 10.55 μl of PCR product was digested with 2.5 Units of *Rsa I* (GibcoBRL, Grand Island ,NY) at 37°C for 8 hours. Digestion products were run in a 3% agarose gel for 1 hour at 73 volts. The gel was stained with ethidium bromide (Sigma Chemical Company, St. Louis, MO) (5 μg/ml) for 30 min and destained in H2O for 30 min.

Description of Polymorphism. The PCR product for this DNA region with high homology to bovine H-FABP is 612 bp (See attached Figure). Two alleles for the DNA region with high homology to the H-FABP gene have been identified when digesting with *Rsa I* (GibcoBRL, Grand Island ,NY). The A allele has two recognition sites which yields fragments of 298, 251 and 63 bp (See attached Figure). The B allele contains only one

recognition site yielding fragments of 361 and 251 bp (See attached Figure). The 63 bp fragment is not visible in the attached Figure.

Mendelian Inheritance. Departure from Mendelian expectation was not seen in 11 full-sib families, where a total number of 158 animals were evaluated. All animals evaluated had at least one parent that was heterozygous for this H-FABP DNA marker polymorphism.

Allele Frequencies. The following Table presents the allele frequencies found in six breeds of cattle. The A allele frequency of all Hereford animals evaluated was 1.0. The Brahman breed exhibited the highest frequency for the B allele of .625. The International Bovine Reference Family Panel (IBRFP) parents were also evaluated for allele frequencies. The IBRFP parents were composed of crossbred and purebred animals descending from both *Bos Indicus* and *Bos Tarus* lines.

Table 1. Allele Frequencies for a DNA Region with High Homology to H-FABP are Shown for Six Breeds of Cattle, and the IBRFP Parents.

Breed	Number	A allele Freq.	B allele Freq.
Holstein	13	96.2%	3.8%
Angus	23	84.8%	15.2%
Gelbvieh	17	85.3%	14.7%
Brahman	4	37.5%	62.5%
Polled Hereford	20	97.5%	2.5%
Hereford	23	100%	0%
IBRFP Parents	42	75%	25%

Chromosomal Location. Linkage analysis utilizing the Cattle Genotypic Database has determined that this DNA region with high homology to the bovine H-FABP gene is on bovine chromosome number 6. This chromosomal location was determined by genotyping IBRFP samples.

Comments. H-FABP is a member of a large class of proteins called fatty acid binding proteins which are believed to be important in the transportation of hydrophobic lipids within the bloodstream (Peeters et al., 1989). Two RFLPs have been found in bovine for this class of proteins. Barendse et al. (1991) found a RFLP in bovine DNA digested with

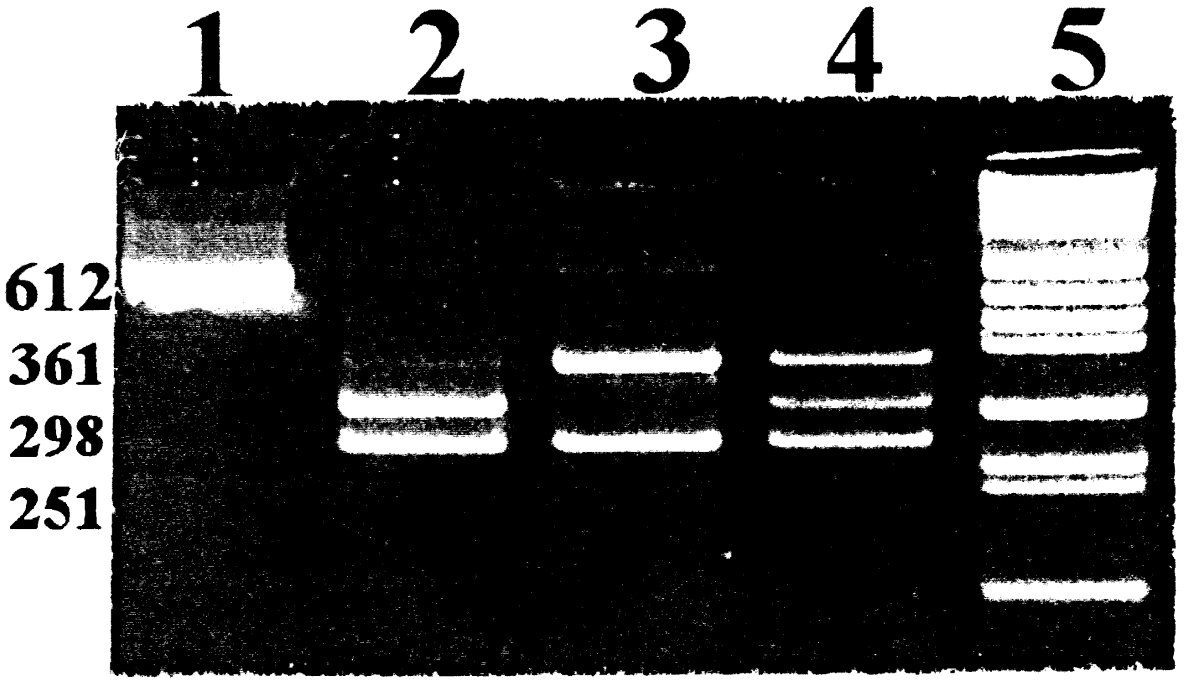
Taq I when probing with the rat intestinal fatty acid binding protein. Another RFLP was detected in the bovine fatty acid binding protein gene with *Pvu II* (Wilson et al., 1989).

The entire 612 bp of this DNA region have been sequenced. Sequence data for this PCR product showed high homology to both bovine H-FABP and to bovine mammary derived growth inhibitor (MDGI). The sequence of this PCR product did not match either of the cDNA sequences for these two genes. Therefore, it is possible that this amplification product is a pseudogene for either H-FABP or MDGI. Until further data is gathered to conclude the true identity of this DNA fragment, it will be referred to as H-FABP within this research.

The H-FABP polymorphism described here could serve as a useful marker when searching for quantitative trait loci in bovine reference families with *Bos Indicus* ancestry. The conditions used to amplify the bovine H-FABP marker produced a PCR product of expected size for both ovine and murine samples. No PCR product was attained for porcine samples.

Figure Legend

Figure 1. Genotypes for a DNA region with high homology to H-FABP on a 4% agarose gel. Lane 1: Uncut PCR product (612 bp); Lane 2: AA Genotype (298 and 251 bp); Lane 3: BB Genotype (361 and 251 bp); Lane 4: AB Genotype (361, 298 and 251 bp); Lane 5: Boehringer-Mannheim (Indianapolis, IN) Marker VI. The smear at the top is unseparated bands of 2176, 1766, 1230, and 1033 bp. The remaining band sizes in descending order are 653, 517, 453, 394, 298, 234, 220, and 154 bp.



APPENDIX N--SSCP PROTOCOL

1) Perform a normal optimized PCR with a total volume of 15 µl or more.

2) Pour a 4 - 12% gradient acrylamide (Fisher Scientific, Fair Lawn, NJ) gel (4%top, 12% bottom) and allow to set.

3) When you are ready to run your gel remove 12 µl of PCR product, add 5 µl of 15% Ficoll (Sigma Chemical Company, St. Louis, MO) dye and 1 µl of Methylmercury (II) hydroxide, 1 M in water (Johnson Malthey Catalog Company, Ward Hill, Mass.) (follow necessary precautions).
   ** DO ALL WORK IN FUME HOOD, AND WEAR PROTECTIVE CLOTHING AND MASK.

4) Heat all samples for 4 minutes at 85° C.

5) Immediately place all samples on ice when they are done heating.

6) Load samples immediately into wells on the 4 - 12% gradient gel.
   **DISCARD ALL WASTE IN CLEARLY MARKED CONTAINERS.

7) Running time and temperature during the run will vary with samples. A 10° C running temperature is recommended which can be maintained with a recirculator for the inner, outer or both chambers of the acrylamide gel box. Standard running times of 8 hours at 300 Volts were used for SSCP work with digested LPL PCR products and intact H-FABP PCR products.

8) When the run is over shut off the recirculator and remove your gel from the glass plates and place on seran wrap. Stain the gel for 30 minutes in Ethidium Bromide (5 µg/ml; Sigma Chemical Company, St. Louis, MO) and destain in ddH20 for at least 15 minutes. Take a picture of gel under UV light.

VITA

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