ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE BETA-2-ADRENERGIC RECEPTOR GENE WITH GROWTH, CARCASS AND MEAT CHARACTERISTICS IN BEEF STEERS AND HEIFERS

By

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STATEMENT BY AUTHOR

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DEDICATION

To my wife Peruth Muumba, my sons John Muumba and Aroun Karakata for their love, passion, patience and their understanding towards my desire to pursue graduate studies.

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ABSTRACT

A total of 693 beef steers and heifers of six breeds were utilized in an association study. Traits analyzed were birth weight (BW), weaning age (WA), adjusted weaning weight (WW), hot carcass weight (HCW), marbling score (MS), 12^{th} rib fat thickness (FT), Longissimus dorsi muscle area (LM), estimated kidney, pelvic and heart fat (KPH); and yield grade (YG). Five single nucleotide polymorphisms (SNPs) in the beta-2 adrenergic receptor (ADRB2) gene were evaluated as markers for beef traits. One SNP was uninformative and was discarded. There was an interaction of gender by A11C SNP on WA ($P<0.05$), WW ($P=0.1$) and KPH ($P=0.05$). An interaction was detected between the C1027T SNP and gender on WA ($P<0.05$) and WW ($P<0.1$). There was no association ($P>0.1$) between A11C, C41T and C1027T SNP's with the remaining traits. These results suggest that SNP's for ADRB2 gene might be useful for WA, WW and KPH traits.

CHAPTER 1

INTRODUCTION

Domestic animals are bred to improve their useful attributes. For beef cattle the primary attribute is to achieve rapid growth and therefore slaughter weight within the shortest time. Great importance is also paid by both producers and consumers to higher quality meat which is tender and has a desirable amount of fat.

Selection of beef animals with higher growth rate and better carcass composition is of great significance to consumers and breeders. For a long period of time, selection programs have relied on phenotypic traits. There exists a contradicting body of knowledge from different literature sources regarding heritability for different phenotypic traits in beef cattle. Specifically, heritability estimates of these traits are quite variable as shown in table 1.1 below from Koch et al., 1982.

Gregory et al., 1995 reported heritability to be similar among all breed groups combined, pure breeds combined, and composites combined, for growth, carcass and meat traits. The heritability values were not necessarily the same for a given group type of animals indicating variation in heritability for the reported phenotypic traits. Utrera and Vleck, 2004 reviewed seventy two papers published from 1962 to 2004, which reported estimates of heritability for carcass traits. They concluded that heritability estimates for most traits varied greatly, and this could be explained by differences in breed groups, method of estimation, effects in the model, number of records, measurement errors, gender and management. Although environmental factors play a role in influencing the phenotypic characteristics, there is a clear genetic influence underlying the performance

of a given breed of animals. Understanding genetic polymorphisms underlying different phenotypic traits in beef cattle, together with their interaction with the environment can assist in selection programs.

Different approaches exist for maximizing genetic progress. The oldest of these involves selecting cattle with desirable traits and selecting against those presenting with undesirable traits. Recently, ultrasonic measures of body composition have become accurate enough for genetic evaluation along with the relationships to carcass traits, (Wilson et al., 1998). Another process termed dynamic mate selection whereby candidates are selected and mated by considering additive and non additive genetic effects has also been introduced as a method to improve genetic quality of beef animals.. The ultimate objective of this approach is to maximize genetic merit in some future generation such as grand progeny.

Reproductive technologies have become an important component of improving the rate of genetic progress by their impact on generation interval. Reducing the generation interval increases the rate of genetic progress. Reproductive technologies include in vitro embryo production, embryo splitting, embryo sexing, cloning, semen sexing and same sex mating. Of all the potential reproductive technologies, artificial insemination and embryo transfer remain the two techniques that are of practical, though limited use, in beef breeding programs today and in the nearby future (Miller 2002).

Genetic markers and quantitative trait loci (QTL) became a practical reality in beef breeding programs in 2000 with the introduction of a marker associated with marbling by Genetic solutions Pty limited Australia. This type of approach is of merit for traits that

are difficult to measure or which have low heritability. Marker assisted selection (MAS) allows for the accurate selection of specific DNA variations that have been associated with a measurable difference or effect on complex traits. Markers for complex traits such as marbling are associated with only one of the many genes that contribute towards that trait. The presence or absence of numerous other unmarked genes and the production environment will determine whether an animal actually displays the desirable phenotype. MAS should be seen as a tool to assist with, and not as a replacement for traditional selection techniques. MAS selection is of potential benefits for traits that have low heritability, are difficult or expensive to measure, cannot be measured until the animal has contributed to the next generation (carcass traits), are currently not selected for as they are not routinely measured (tenderness), and are genetically correlated with a trait that you do not want to increase (most likely associated genes affects one trait of the pair but not the other e.g. affects marbling but not back fat. This will gradually lead to increase in a herd or population of beef cattle of desirable meat and carcass traits (Eenennaam, 2007).

The commercially available markers for carcass quality traits that have been validated by the National Beef Cattle Evaluation Consortium (NBCEC) include GeneSTAR® Quality grade (http://www.bovigen.com) which is comprised of four markers (QG1 (TG5), QG2 (M2), QG3, QG4) that are associated with marbling score and quality grade (percentage choice and prime). The TG5 DNA variation is in the 5' leader sequence of the thyroglobulin gene (Eenennaam, 2007).

Igenity TenderGENE ™ (http:// www.igenity.com) is a DNA genetic marker panel comporised of three markers (UoGCAST1, Calpain 4751 and calpain 316). An increase in tenderness is associated with substituting a "C" allele at calpastatin (UoGCAST1) and a "C" allele at both μ–calpain loci (Calpain 4751 and calpain 316). This gene produces an enzyme which weakens muscle fibers thus increasing tenderness during the postmortem aging process (Eenennaam, 2007).

GeneSTAR® Tenderness (http:// www.bovigen.com) is a DNA genetic panel test compised of three markers (CAST-T1, calpain 316-T2, and Calpain 4751-T3). An increase in tenderness is associated with substituting a "T" allele at calpastatin (CAST-T1) and a "C" allele at both μ–calpain loci (Calpain 316-T2, and Calpain 4751-T3 (Eenennaam, 2007).

Candidate genes for SNP genotyping beef and milk characteristics

A SNP is just a single base change in a DNA sequence, with a usual alternative of two possible nucleotides at a given position. For such a base position with sequence alternatives to be considered a SNP, it is considered that the least frequent allele should have a frequency of 1% or greater. Although there is a chance for any of the four bases to occur at each position of a sequence stretch, SNPs are usually biallelic in practice. Mutation mechanisms leading to SNP can result either in transitions: purine - purine (A-G) or pyrimidine - pyrimidine (C-T) exchanges or transversions: purine - pyrimidine or pyrimidine - purine (A-C, A-T, G-C, and G-T) exchanges. With twice as many possible transversions than transitions, the transitions over transversion ratio should be 0.5 if mutations are random (Vignal et al., 2002).

Several SNP genotyping studies involving the association of different candidate genes to different production characteristics (carcass and meat characteristics, milk characteristics) have been done in different species and different breeds of animals. In pigs, Pituitary specific transcription factor (PIT1) genotypes been associated with fat thickness, Expected Progeny Difference (EPD) for fat thickness (Franco et al., 2005), growth and carcass composition in crosses between European wild boar and Pietrain pigs(Brunsch et al., 2002), whereas in Angus beef cattle PIT1 polymorphisms were not associated with growth and carcass traits (Zhao et al., 2004). Growth Hormone (GH) polymorphism in pigs has been associated with fat thickness and average daily gain (Franco et al., 2005), whereas in Beijing Holstein cows, they have been associated with milk yield and milk fat on different lactation stages (Zhou et al., 2005). Growth Hormone Releasing Hormone (GHRH) polymorphisms in pigs have been associated with the average daily gain and EPD for fat thickness (Franco et al., 2005) whereas in cattle significant association were observed between cold carcass weight and longissimus muscle area in Korean cattle (Cheong et al., 2006).

SNP (a G to C substitution) in the calpastatin gene that encodes calpastatin enzyme known to regulate postmortem proteolysis, has been associated with increased longissimus muscle tenderness across days of postmortem aging and significant reduction in the percentage of steaks rated unacceptably tough by consumers based on an assumed threshold level (Schenkel et al., 2006). In beef cattle, a SNP in the leptin (obese) gene that encodes the leptin hormone synthesized in adipocytes and known to regulate long

term body fat weight and composition, has been associated with lean yield, fatness (fat yield and subcutaneous fat), and tenderness (Schenkel et al., 2005).

Milking characteristics have also been associated with different SNP genotypes in different candidate genes in dairy cattle. Brym et al., 2005 found a significant association for a SNP in the prolactin gene and milk yield and fat content in black and white cows. Significant associations have also been found between ADRB2 genotypes in Holstein bulls and milk production, milking characteristics and milking rate in respective daughters (Du et al.,)

In order for a candidate gene to be included in MAS for beef cattle, there should be a link between its effect to carcass and meat characteristics. The ADRB2 gene is thought to be one of such candidate genes as evidenced from different studies on membrane densities, gene expression and SNP genotyping of ADRB2 to different cattle production traits. For example, an association between adrenoceptor concentrations and milkability was described by Roets et al., 1986. Subsequently Roets et al., 1989, found a significant correlation between the ratio of β_2 to α_2 adrenoceptors on cell membranes when investigating the relationship between numbers of α_2 and β_2 in teat tissues and milkability of primiparous cows. Further more Roets et al., 1995 described a relationship between numbers of β_2 and α_2 on blood cells of bulls to milkability of their daughters.

Valero et al., 2002 described a negative correlation between β_2 –AR mRNA levels to milking duration and milk removal rate. Subsequently Inderwies et al., 2003 describes a positive correlation between milkability and α_2-AR mRNA expression following α-AR stimulation whereas during β-AR stimulation no correlations were detected.

Subsequently a SNP at position 178 in the ADRB2 gene that consists of either a cytosine or a thymine that did not change the amino acid sequence in the encoded protein has been analyzed. This SNP was found to be placed outside the quantitative locus peak for marbling; however a peak for back fat existed at that position (Schimpf et al., 2001). The objective of this study was to evaluate SNPs (A11C, C41T, A-408C, A468G and C1027T) in the ADRB2 gene and how they relate to different performance traits in beef calves.

Table 1.1. Heritability estimates from several literature sources (Koch et al., 1982)

	Eight different literature sources								
Item	1	2	3	4	5	6	7	8	Avg
BW						.45			.45
Gain to weaning						.30			.30
Feed lot gain						.34			.34
Carcass weight	.57	.39	.56				.68	.54	.55
Retail product									
Weight			.64		.38		.38	.55	.52
Percentage		.40	.28		.66			.49	.41
Fat trim weight			.46	.50	.39		.94		.57
Bone weight			.38				.56		.47
Kidney fat weight				.72					.72
FT	.24	.43	.50	.43	.57		.68	.50	.48
LM	.26	.73	.41	.40	.25		.28	.45	.40
Marbling	.17	.62	.31	.73	.31		.34	.56	.42

CHAPTER 2

LITERATURE REVIEW

General overview on adrenergic system involvement in growth regulation

Epinephrine and norepinephrine represent the physiological agonists for the adrenergic system. Norepinephrine, the catecholamine for the sympathetic nervous system neurotransmitter molecule, is biosynthesized from tyrosine; it also circulates in the serum at relatively high concentrations. The catecholamine epinephrine is biosynthesized from norepinephrine and is the methylation product of norepinephrine; secreted from the adrenal medulla, it circulates at lower concentrations than norepinephrine in most mammalian species, but during stress it usually responds to a greater response than norpinephrine (Mersmann, 1998).

Norepinephrine and epinephrine are inactivated by catechol-o-methyl transferase, an enzyme that methylates the catechol-ring hydroxyl groups, and by monoamine oxidase, an enzyme that deaminates the ligand, Following release from sympathetic nerve endings, norepinephrine may be reabsorbed by specific reuptake mechanisms (at synaptic clefs and myoneural junctions) to decrease the concentration at the effector site (Mersmann, J.,1998).

Rate of growth, muscle mass, and meat quality are becoming increasingly important economic traits in food producing animals. A number of drugs that activate β_2-ARs (β-agonists) are known to have profound effect on these traits (Hoey et al., 1995). The best studied synthetic β -AR agonists include clenbuterol, cimaterol, ractopamine and L-644,969. These drugs have various effects on nutrient metabolism that lead to increased

lean and decreased fat deposition in meat animals and other species. Their effects well pronounced in ruminants and confined to skeletal muscles (NRC, 1994). Mode of action of β-AA on β_1 and β2 receptors is to increase protein synthesis (Bergen et al., 1989) and in abundance of mRNA for specific proteins such as myosin and actin (Grant et al., 1993; Smith et al., 1989). Others suggest that most of the protein accretion in muscles if not all is due to reduced protein degradation (Bohorov et al., 1987, Dawson et al., 1991), possibly mediated by reduced activity of calpains and other specific proteolytic systems such as Cathepsin B, Cathepsin L plus Ca^{2+} dependent protease (Wang and Beermann, 1988; Meyer, 2001).

The β -AR receptors control a large number of different physiological functions in various body tissues and organs. This complex mechanism of action of β-ARs suggests that the mechanisms for the observed changes in the carcass traits might be complex. Any proposed mechanism must begin with the possibility of direct effects of the agonist on skeletal muscle and adipocyte. However, many other mechanisms, such as modification of blood flow, release of hormones, or central nervous system control of feed intake may contribute to the overall effects observed with a given β-AR agonist in a given species (Mersmann, 1998).

Direct effects of β_2-AR agonists on muscle tissue have been studied. Byrem et al., 1997 observed 65% net protein accretion in vivo, on the basis of amino acid uptake, when cimaterol was infused for 21days in growing steers into a single hind limb as compared to the contralateral limb infused with saline. This anabolic response was observed to be transient by the fact that it peaked up at 14 d, but was greatly attenuated by 21 d of

treatment. Similar effects were observed in female rats treated with clenbuterol, which caused muscular hypertrophy, accompanied by reduction in β_2-AR density in the heart and hind limb (Sillence et al., 1991) This might be a result of β-AR desensitization (Hausdorff et al., 1990) or reduction in β-AR density as observed in longissimus dorsi muscle of pigs treated with ractopamine for 3 weeks (Sainz et al., 1993). On the other hand, growing pigs when treated with ractopamine, showed appreciable increases in body protein deposition (NRC, 1994)

However, under normal conditions, in cattle not treated with β-AR agonists, no correlation was found between β-AR densities with growth, carcass and meat characteristics indicating this was a less useful predictor of performance traits in beef cattle before slaughter (Hoey et al., 1995). Assays in which β-ARs functions are measured, such as SNPs analysis, instead of their density, may be a more appropriate predictor of performance traits in beef calves.

General overview on adrenergic receptors (ARs)

The physiological effects of adrenergic system in different tissues are mediated by binding of catecholamines to adrenergic receptors (ARs) in various tissues. In 1948 ARs were first divided into two distinct types, alpha (α) and beta (β) ARs. By the use of physiological parameters, these were defined by their relative responsiveness to a series of sympathomimetic amines. The α-AR was characterized by a potency series of epinephrine>norepinephrine>>isoproterenol, whereas the β-AR has the potency series isopreteronol>epinephrine>noepinephrine (Ahlquist, 1948). Both norepinephrine and epinephrine stimulate the two receptors. Epinephrine is more potent (effective at lower

concentrations) to α-AR than norepinephrine. Adrenergic receptors belong to a family of G coupled receptors which are located in the cell membrane. The primary structure of both alpha and beta receptor is remarkably similar. They both contain seven relatively hydrophobic transmembrane domains that anchor the receptor in the plasma membrane. There are four exracellular portions on the outside of the membrane (three loops connect adjacent transmembrane domains) and four intracellular portions on the inside of the membrane (three loops connect adjacent transmembrane domains. The ligand binding site is in the center of the seven transmembrane domains and involves amino acids from several of the domains. The site of interaction with the Gs protein have been localized to portions of intercellular loops 2, 3 and 4 (Gether , 2000)

Pharmacologically α and β ARs are classified into the receptor types α_1 and α_2, and β_1, β_2 and β_3, respectively (Civantos et al., 2001), with norepinephrine being more potent for the β_1-AR than for β_2-AR (Mersmann 1998). Discovery of both β_1-AR and β_2-AR allowed better comprehension of adrenergic system functions and it also led to the establishment of prototypical tissues i.e. tissue that predominantly has a single adrenergic receptor subtype as evidenced by its response in the classification studies. Examples of prototypical tissues are heart rat for β_1-AR responses and guinea pig tracheal musculature for β_2-AR. Prototypical tissues assisted in classification of large numbers of agonists and antagonists regarding their specificity for the receptor subtypes (Mersmann 1998). Studies of rat adipocyte function and ligand binding demonstrated the evidence of β_3-AR that had specific agonists different from β_1-AR and β_2-AR (Arch and Kaumann, 1993).

An interesting characteristic of the β_3-AR is that several antagonists for β_1-AR and β_2-AR are partial or in some cases full antagonists for the β_3-AR (Lafontan and Berlan, 1993).

Both β_1- and β_2-ARs stimulate the membrane bound enzyme adenylate cyclase; this action leads to the accumulation of adenosine3':5'-cyclic phosphate (cAMP), the second messenger of β-adrenergic action. In some systems α_2-adrenergic receptors inhibit adenylate cyclase, whereas α_1-ARs are not clearly linked to the adenylate cyclase system. The β-ARs are highly studied due to a number of reasons. The first is their ubiquity; they are found in virtually all mammalian tissues. The second is their close coupling to the adenylate cyclase. Finally their blockade or stimulation, has obvious and important clinical implications relevant to the therapy of a wide variety of prevalent human diseases (Stiles et al., 1984)

β -AR amino acid sequences vary across different tissues in the same specie, across different species and across similar tissues in different species. In a given species, the β_1-AR, β_2-AR, and β_3-AR have approximately 40-50% homology with each other, A given β-AR subtype has approximately 75% or greater homology across species. For example, the bovine β_3-AR has approximately 85%amino acid sequence homology with human β_3-AR and 75% homology with rat and mouse β_3-AR (Pietri-Rouxel and Strosberg., 1995).

From molecular cloning studies, α_1-AR was originally classified into α_{1AD}, α_{1B} and $\alpha_{1C.}$ (Garcia-Saiz et al. 1992), whereas α2-AR was classified into α_{2A}, α_{2B}, α_{2C} and α_{2D} (Kurose et al., 1994).The α_{2A} in human and porcine and the α_{2D} in rat, murine and bovine, differ in one amino acid only (Hieble et al., 1995). This has led to a combination of both α_{2A} and α_{2B} adrenergic receptor subtypes into one receptor subtype α_{2AD}, as they seem to

be variants of the same adrenergic receptor subtype between species (Venkataraman et al., 1997).

Recent human pharmacological research has subdivided α_1 into α_{1A} (formerly α_{1C}), α_{1B} and α_{1D} (formerly α_{1AD}), whereas α_2 sub classification remained subdivided into α_{2AD}, α_{2B} and α_{2C} (Civantos et al., 2001). α_1-AR are present in many tissues including brain, heart, smooth muscles, liver, spleen and presynaptic nerve terminals, where they mediate a variety of effects including vasoconstriction, glycogenolysis, cardiac inotropy and chronotropry. α_2-ARs are present in a variety of tissues including platelet, brain, vas deferens, spleen, lung, heart, liver, kidney, blood vessels, pancreatic islets and gastrointestinal tract. α_2-ARs have been shown to mediate platelet aggregation, neurotransmitter release, vasoconstriction, regulation of ion secretion and inhibition of insulin secretion (Lomasney et al., 1991)

Role of Adrenergic Receptors on nutrient partitioning

β-ARs have multiple actions on various aspects of nutrient metabolism that lead to increased lean and decreased fat deposition in meat animals and other species (Bell et al., 1988). Their effects are more pronounced in ruminants than they are in other species. More so, the effects seem to be directed more towards skeletal muscles than any other tissues (NRC, 1994). It is also clear that the effects on muscle protein metabolism are mediated directly through binding of the agonist to specific β_1- and β_2- ARs in muscles (Bell et al., 1988).

As mentioned earlier in this review, β-AAs mediate their effects on β1 and β2 receptors either through increasing protein synthesis (Bergen et al., 1989) and in abundance of

mRNA for specific proteins (Grant et al., 1993; Smith et al., 1989) or through reduced protein degradation (Bohorov et al., 1987, Dawson et al., 1991), possibly mediated by reduced activity of calpains and other specific proteolytic systems (Wang and Beermann, 1988).

β-ARs effects on lean tissue protein accretion are more appreciable in ruminants and swine approaching market weight, when the capacity for lean growth is waning and the propensity for fattening is markedly increasing (Carr et al., 1997). As such β-AAs treatments may be of value in feed lot cattle approaching market weight. To date, there are only a few definitive studies on the role of different adrenergic subtypes on growth regulation.

ARs and Milking characteristics

ARs have been studied on blood cells of bulls to predict milking characteristics of their respective daughters. Numbers of α_{2-} and β_2 –ARs on blood cells of bulls have been correlated to milkability of their daughters. AR densities determined on platelet and mononuclear membranes and therefore expressed as fmol radioligand bound per mg of protein revealed no correlation between α_{2-} and β_2-AR densities on blood cells and milkability. However, significant correlation was found with the ratio of β_2- to α_2-AR densities on blood cells and milkability. Good milkability in these experiments appeared to be associated with a high value of this ratio (Roets et al., 1989). Mean flow rate was highly correlated with α_2-AR densities on blood platelet membranes whereas no correlation was found with β_2-AR on intact mononuclear leucocytes (Roets et al., 1995).

In the mammary gland α_1-, α_2- and β-AR have been found mainly in the milk purging system and hardly in the parenchyma (synthetic and secretory tissue). α-adrenergic system probably inhibits milk removal through vasoconstriction whereas β-adrenergic system enhances milk flow through relaxation of teat sphincter, teat wall and large mammary ducts. Binding studies have shown that densities of α_1- and α_2- have been shown to decrease from the teat to the mammary ducts to the parenchyma, whereas density of β-AR remained the same in the teat and large mammary duct, but much lower in the parenchyma. Most of the α_1- and α_2–AR receptors were found in the teat wall, whereas in the parenchyma α-ARs were absent or barely detectable. The β-ARs were similar in the teat wall and the large mammary ducts, but much lower in the parenchyma. (Hammon et al., 1993).Milk yield and peak flow rates are controlled mainly by α- and β-ARs in the region of the large ducts in the mammary gland. Milk yield and peak flow rates following amputation of the distal part of the teat, including the teat canal and teat sphincter were not affected when compared before and after the operation (Inderwies et al., 2002)

The expression of ARs in the mammary gland has also been quantitatively studied through mRNA expression for a given receptor. The mRNA transcripts of all α-AR subtypes except α_1D, have been quantitatively measured in mammary gland with α_{1A} being the highest, followed by α_{1B}. For the α_2-AR, α_{2AD} was found to be the highest, followed by α_{2B} and then α_{2c}. Within the β-ARs, the β_2 receptor type was highly expressed followed by β_1 and β_3 (Inderwies et al., 2003; Wellnitz et al., 2001). The mRNA levels determined from Holstein blood also suggest that milking duration and

milk removal rate are associated with amount of α_2-and β_2-AR mRNA expression levels. A study involving eight dairy cows evaluating milking duration and milk removal rate demonstrated a positive and negative correlation, respectively, with α_2–AR. In contrast, milking duration and milk removal rate had a negative correlation, respectively with β_2 – AR mRNA levels (Valero et al., 2002).

It is clear that ARs have a potential role in predicting calves performances from their sires and can also be associated to some of the performance traits. Since metabolism is a well coordinated process, it is probably true that some of the effects associated with ARs density and mRNA levels expressions on milk characteristics may also be related to mechanisms through which β-AAs regulate nutrient partitioning between lean and fat in skeletal muscles of feedlot cattle.

SNP genotyping techniques

Several techniques have been used in SNP genotyping. Apart from those techniques based on direct hybridization, the other techniques involve generation of allele specific molecular reaction products and separation and detection of the allele specific products for their identification. For microsatellite markers, there is a standard procedure for genotyping, involving PCR and size determination of the amplified fragment by acrylamide gel electrophoresis (Vignal et al., 2002)

Most techniques that have been used in genotyping SNPs involving studies on carcass and meat characteristics involve generation and separation of allele specific product. For unknown SNPs, cloning and sequencing of candidate genes whose product play a role in cellular or metabolic processes, such as energy homeostasis and lipolysis has been

considered. Signaling molecules and their receptors can also be considered as candidates for genes involved in meat traits. In addition, genes whose effects in other species on carcass traits have been described in the literature can be considered. The sequence information, obtained by cloning and sequencing of the PCR fragments, is then used as an initial step in a polymorphism screening by comparing the data with the sequence in NCBI database (Haegeman et al., 2003)

If the SNP to be studied involves a restriction enzyme site, PCR-RFLP is used as the genotyping technique. Candidate genes whose SNP do not involve a restriction enzyme site have been studied by Single strand Conformational Polymorphism (SSCP). SSCP are based on the fact that one single base difference in DNA fragments of up to 300 bp will usually change the conformation in the way that can be detected by non denaturing poly-acrylamide gel electrophoresis (Haegeman et al., 2003).

TaqMan® SNP genotyping

The TaqMan® SNP genotyping assay consists of a single tube which contains forward and reverse primers for amplifying the polymorphic sequences of interest, and the two TaqMan® MGB probes for distinguishing between the two alleles. Each of the two TaqMan® MGB probes has a reporter dye at the 5' end of each probe labeled differently, either with VIC® dye or FAM™ dye linked to the 5'end of each of the two alleles. A minor groove binder (MGB) at the 3'end of each probe provides a modification that increases the melting temperature (T_m) for a given probe length, which allows the design of shorter probes. Shorter probes results in greater differences in values between matched and mismatched probes, producing robust allele discrimination. The presence of a non

fluorescent quencher (NFQ) at the 3' end of each probe ensures accurate measurement of the reporter dye by Real time PCR system.

Each of the two TaqMan® SNP MGB probe binds specifically to its complementary sequence between the reverse and the forward primers. Extension of primers bound to the template DNA by AmpliTaq Gold® DNA polymerase, results in cleavage of probes that are only hybridized to the target. Cleavage results into reporter and quencher dye separation, leading to increased fluorescence by the reporter dye. The fluorescence signal is transmitted to the Sequence Detection System (SDS) Software. The SDS uses the fluorescence measurements made during the plate read to plot Fluorescence (Rn) values based on the signals from each well. The plotted fluorescence signals indicate which alleles are in each sample. A substantial increase in VIC – dye fluorescence only indicates homozygosity for allele one, increase in FAM-dye fluorescence only indicates homozygosity for the other allele whereas fluorescence from both VIC and FAM – dyes indicates heterozygosity for the two alleles.

TaqMan® SNP genotyping has been applied in carcass and meat characteristics association studies to candidate genes. TaqMan® SNP genotyping of the GH gene and transcription factors of GH in a large population of Brahman steers suggested that sire was a significant source of variation in average daily gain, carcass yield and marbling score. However, polymorphisms in the GH gene and its transcriptional regulators were not informative predictors of growth and carcass characteristics in Brahman steers (Beauchemin et al., 2006). TaqMan® SNP genotyping of the GH1 gene in Angus and

short horn Australian feedlot cattle showed association with intramuscular and rump fat distribution (Barendse et al., 2006)

Beef Grading

In order that one can understand the beef performance traits described in this study, it is felt important to review the USA beef grading system. The beef grading system in the United States is an attempt to connect physical carcass traits with quality such as palatability (tenderness, juiciness and flavor). Grading systems are designed to recognize value differences among individual carcasses. The grading systems provide premiums and discounts based on measures of carcass quality. Severe discounts are usually applied to non-conforming carcasses. Breeding, feeding and management programs can be targeted to exploit specific grids. The beef grades should not be confused to routine meat inspection that entails to inspect carcasses and meat for consumer safety. In the United States two types of grades are recognized; quality grades and yield grades. Beef carcasses may carry a quality grade, a yield grade or both a quality and yield grade (Burson, 2004)

Quality grades

Quality grades provide an indication of expected palatability or eating satisfaction of the meat. Two important variables, i.e. marbling and maturity determine quality grades.

Marbling is defined as the flecks of intramuscular fat distributed in muscle tissue. It is usually evaluated in the LM between the 12^{th} and 13^{th} ribs. Marbling is the primary determinant of the quality grade. It has been associated with eating quality particularly juiciness and flavor but not necessarily tenderness. Amount of marbling in the eye muscle is divided into ten degrees as shown from lowest to highest: devoid, practically devoid,

traces, slight, small, modest, moderate, slightly abundant, moderately abundant and abundant. In addition to marbling, when the quality of the muscle is of inferior quality, the quality grade is further adjusted down due to dark color lean, course texture and softling. Desirable LM will exhibit an adequate amount of finely dispersed marbling in a firm, fine textured, bright cherry-red colored lean. As the animal matures, the characteristics of the muscle changes, and muscle color becomes darker, and muscle texture become coarser.

Maturity

Maturity is estimated visually by cartilage ossification (hardening of the cartilage into bone), rib bone shapes, lean color and texture. During early stages of growth, ossification starts in the hind quarters (sacral and lumbar vertebrae) and progresses towards the fore quarter (thoracic vertebrae) with advancing age. However, approximate chronological age groupings for maturity are shown in table 2.1.

Carcass maturity is first determined from its skeletal characteristics and adjustments made according to the lean characteristics. In young beef carcasses the lean flesh is light cherry red in color and fine in texture. With advanced maturity the flesh color becomes darker and coarser in texture.

Depending marbling and maturity the final quality grades assigned to the carcass may either be prime, choice, select, standard, commercial, utility, cutter and canner. Quality grading is voluntary and therefore not all carcasses are quality graded. Parkers may apply their own “house brand” to merchandise their beef. Carcasses merchandised as ungraded beef usually are those that do not grade prime or choice. They are generally termed “No

Roll" by the industry, because a grade stamp has not been rolled on the carcass (Burson, 2004).

Figure 2.1 assumes that firmness of lean is comparably developed with the degree of marbling and that the carcass is not a darker cutter. Maturity increases from left to right (A through E). The A maturity figure is the only portion applicable to bullock carcass

Yield grade

(Hale, 2004) describes YG as an estimate of the percentage closely trimmed (1/4 inch fat or less) boneless retail cuts derived from the major whole sale cuts (round, loin, rib and chuck) of a carcass. It is based on a scale of 1 to 5 where 1 is the highest yield and 5 is the lowest yield of cutability. YG takes into account of adjusted FT, percentage KPH, HCW and LM.

FT is the amount of external fat at the 12^{th} rib as measured by FT three-fourths the length of the LM from the chine. This value can be adjusted to reflect the unusual amount of fat in other areas of the carcass. HCW reflects the weight of unchilled carcass in pounds, taken on the slaughter - dressing floor shortly after slaughter. The amount of KPH fat is evaluated subjectively and is expressed as the percentage of carcass (this usually will be from 2 to 4 percent of carcass weight). The LM is determined by measuring the size in inches, using a dot grid) of the LM at the 12^{th} rib.

Therefore YG = 2.50 + (2.50xadjusted FT, inches) + (0.20xpercent KPH) + (0.0038x HCW, pounds) – (0.32xLM, square inches).

Justification

As reviewed before, β_2 adrenergic receptors just like the β_1 receptors are ubiquitous in almost all body tissues where they play specific roles. In the mammary gland where they control milk yield and peak flow rates, they are found in the teat wall and large mammary ducts and less in the parenchyma (Hammon et al., 1993). In skeletal muscles both β_1 and β_2 receptors are thought to play a role in nutrient portioning. β-AR agonists like clenbuterol, cimaterol, ractopamine and L-644,969 have multiple actions on various aspects of nutrient metabolism that lead to increased lean and decreased fat deposition in meat animals and other species. Mode of action of β-AA on β_1 and β2 receptors is to increase protein synthesis (Bergen et al., 1989) and in abundance of mRNA for specific proteins (Grant et al., 1993; Smith et al., 1989). Others suggest that most of the protein accretion in muscles if not all is due to reduced protein degradation (Bohorov et al., 1987, Dawson et al., 1991), possibly mediated by reduced activity of calpains and other specific proteolytic systems (Wang and Beermann, 1988).

Already the Waguli cross bred cattle from V bar V Ranch have been reported to posses more marbling than the average crossbreed that is common to the Southwest. Majority of these carcasses graded high choice and prime and their marbling scores ranged from moderate to abundant (Marchello et al.,)

It is therefore hypothesized that the ADRB2 gene in cross breed cattle at V bar V Ranch may play a role influencing nutrient partitioning function between lean and fat. This study was therefore done to analyze different SNPs in the ADRB2 gene and their association to performance traits in beef calves.

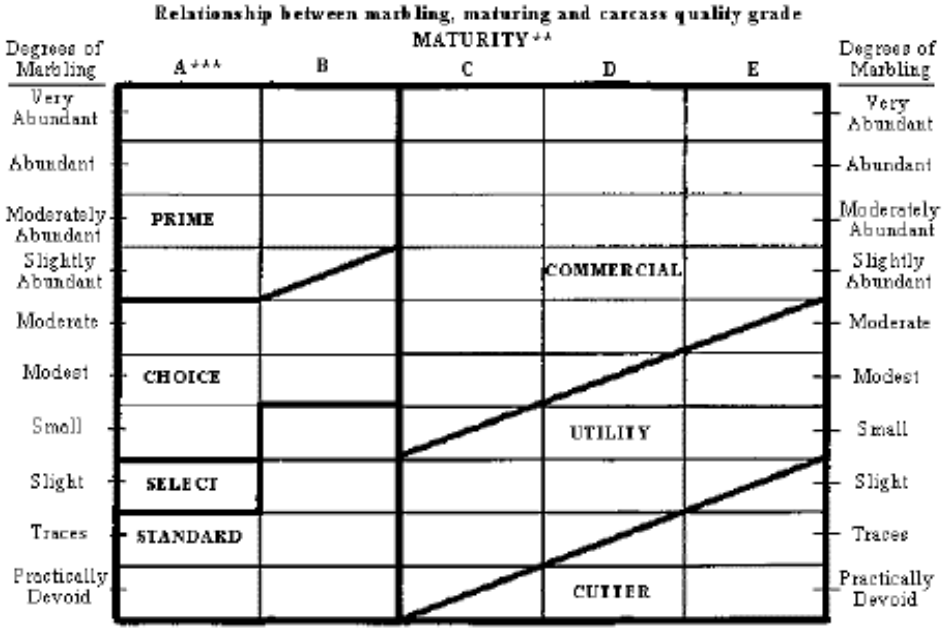
Objectives

The overall objective of this study was to determine the association between single nucleotide polymorphism (SNP) in the ADRB2 gene to growth, carcass and meat characteristics in beef calves. The specific objectives were to determine frequencies of different SNP alleles and their genotypes in the ADRB2 gene in beef calves and the association of these genotypes to growth, carcass and meat characteristics.

Table 2.1. Guidelines for determining skeletal maturity for immature beef calves

Maturity	Sacral Vertebrae	Lumbar Vertebrae	Thoracic Vertebrae
A	Distinct separation to completely fused	Cartilage evident to nearly completely ossified	No ossification to slight evidence of ossification

Figure 2.1. Relationship between marbling, maturity and carcass quality (Burson, 2004)



CHAPTER 3

MATERIAL AND METHODS

Animals and sample collection

Six hundred and ninety seven Crossbreed beef calves of gender identified as steers, bulls or heifers from V bar V beef ranch were used in the study. These animals represent the calf populations from the V-V ranch for the years 2001 to 2005.The breeds involved ranged from Angus Cross, Red Angus Cross, Beefmaster Cross, Purebred Hereford, Hereford Cross, and Waguli Cross. All animals were born and raised to finishing size at the V Bar V Ranch. The ranch is owned and operated by the University of Arizona (U of A), College of Agriculture. The farm is stocked with four cattle breeds, namely registered and commercial Hereford, Brahman crosses and two composite breeds. All cows are mated to Hereford bulls in a multi-sire system during the farms' breeding season. All bulls used have passed the BSE and their FAA status was determined.

Cattle used in this study were fed at the U of A feedlot and harvested at the U of A Meat Science Laboratory or Sunland Beef (Tolleson, AZ). The Meat Science Laboratory personnel evaluated the carcass 48hr post harvesting for USDA YG and quality grade factors (USDA, 1989). Factors for YG included HCW, LM, KPH and FT. HCW and LM are presented on basis of 100 lb HCW. Quality grades were based on MS, maturity (based on lean maturity and skeletal maturity), muscle color, firmness and texture (Figure 2.1 illustrates quality grade procedures). MS was transformed to a scale in which traces of marbling ranged from 300 to 399, slight marbling ranged from 400 to 499, small marbling ranged from 500 to 599 and so on. Also quality grade was transformed to a

scale in which Select grade ranged from 400 to 499, Choice grade ranged from 500 to 599 and Prime grade ranged from 600 t0 699.

The Identity numbers, dams identity, breed, sample type collected from each individual animal, as well as other additional information are recorded. The dams are recorded as a known parent from observation at the birth's offspring. Sires are recorded as a known parent either from the artificial insemination records of the bull, introduction of specific already known bull to run with dams in pastures or through DNA parentage determination. Samples were collected as whole blood by bleeding cattle using a syringe and a vacutainer tube containing an anticoagulant EDTA. Vacutainer tubes were then transported on dry ice to the laboratory for DNA extraction

DNA extraction

DNA was extracted by using a procedures described in Genomic DNA from Blood, User manual, NucleoSpin® Blood (2005). 25 µL of proteinase K and 200 µL of lysis buffer B3 were added to 200µL of whole blood in a 1.5 ml microcentrifuge tube for each particular blood sample. The mixture was then vortexed vigorously for 20 seconds before it was incubated at 70^{o}C for 15 minutes for fresh drawn blood samples. The incubation time with proteinase K was increased to 30 minutes in old blood samples that have been kept at 4 ^{o}C before DNA could be isolated. The DNA binding conditions were adjusted by adding 210 µl of 100% ethanol to each sample tube and vortexing for 10 seconds. To allow DNA binding into the column, the sample was then loaded into NucleoSpin® Blood column placed in a 2 ml centrifuge tube, and centrifuged for 1 minute at 11,000 x g.

The silica membrane was washed in a two step procedure. First, 500 µl of buffer BW was added into each NucleoSpin® Blood column sample placed into a new 2ml collecting tube. The mixture was then centrifuged for 1 minute at 11,000 x g and collecting tubes with flow - through were then discarded. In a second washing step, 600 µl of buffer B5 was added into each NucleoSpin® Blood column placed in a new 2ml collecting tube. The mixture was centrifuged for 1 minute at 11,000 x g and the flow – through was discarded. The silica membrane was dried by placing the NucleoSpin® Blood column back into the collecting tube and centrifuging for 2 minutes at 11,000 x g. By dispensing directly onto the dry silica membrane 100 µl of 70^{o} C prewarmed elution buffer BE and centrifuging for 1 minute at 11,000 x g, highly pure DNA was finally eluted from each of the NucleoSpin® Blood column placed in a 1.5 mL microcentrifuge tube.

The concentration (ng/µl) of the eluted in DNA was then evaluated using Nanodrop® ND-1000 Spectrophotometer before it was further diluted down by Barnstead® nuclease free (DNAse/ RNAse free), pyrogen free water to a final concentration of 2ng/µl.

ADRB2 SNP Genotyping

Some DNA sequence in and around the bovine beta-2-adrenergic receptor (β2-AR) is available in the public genome databases (Genbank accession numbers Z86037, Einspanier et al., Schimpf et al.,) These sequences include the ADRB2 sequence coding region (1257 bases inclusive from the ATG start codon to the TAA stop codon), as well as 223 bases upstream from the ATG start codon and the 550 bases downstream from the TAA stop codon.

Five SNPs in the ADRB2 gene; namely A11C, C41T, A-408C, A468G and C1027T were genotyped by using a procedure described in Custom TaqMan® SNP genotyping assays with some modifications (2006).

Briefly, PCR was done using 2µL of 2ng/µL genomic DNA for each ADRB2 SNP genotyped. The other components of the PCR mix included 3.75µL of 2x Absolute Blue QPCR ROX Mix (Cat # AB-4138/A), 0.2 µL of primer/probe mixture and 2µL of Barnstead® Nuclease free (DNAse/ RNAse free), pyrogen free water in a final volume of 7.95µL run in a 384-Well Clear Optical Reaction Plate- ABI PRISM ™ (part number 4309849), covered with MicroAmp ™ Optical adhesive film, PCR compartible, DNase/ RNase free, (Part Number 4311971).

The reactions were run in a GeneAmp® PCR system 9700 – applied Biosystem thermocycler using the following conditions: Initial denaturation at 95^{o}C for 15 minutes prior to 50 cycles of 95^{o}C for 15 sec, 59.3^{o}C and 60.2^{o}C for 1 min for A11C and C41T SNPs respectively. The annealing temperature for A-408C, A468G, and C1027T ADRB2 SNPs was 61.1^{o}C. This was followed by 58^{o}C for 1 min and then a final extension step at 72^{o}C for 10 minutes. After PCR amplification, an end point plate read and analysis was done using an Applied Biosystems 7900HT sequence detection system – ABI PRISM. Figure 3.1 and figure 3.2 show allele discrimination and end plate allele read respectively. Red dots shows fluorescent signals for VIC (a dye for unsubstituted allele), blue dots indicates fluoresecent signals for FAM (a dye for substituted allele) and green dots shows fluorescence signals for both alleles. Plate read document was analysed, automatic allele calls was done and finally the allele calls were converted into genotypes

into the Microsoft excel sheet. The set of primer pairs with their corresponding annealing temperatures for each of the ADRB2 SNPs genotyped are summarized in Table 3.1.

Statistical analyses

The effect of breed and gender on phenotypic traits reported here was tested using the PROC MIXED procedure of SAS (Version 9.1, SAS Inst., Cary, NC). The model included the fixed effect of a respective phenotypic trait and age of dam (AOD) for BW, WW and WA. For the traits studied during harvest, days on feed (DOF) was used as covariate factor. When evaluating the effect of breed and gender on the phenotypic traits, sire and dam were considered random effects. The effect of ADRB2 SNPs on the studied phenotypic traits was again tested with PROC MIXED procedure of SAS. The model included the fixed effect of the marker (SNP) and gender, sire and dam were considered as random effect. Again, AOD for BW, WW and WA formed the fixed effect whereas DOF for the traits during harvest was used as a covariate factor in the association model. WW for study calves was also adjusted for WA. A-408C marker was not included in the ADRB2 gene association analysis as it was found to be uninformative for being 100% homozygous for CC genotype. C41T and A468G were analyzed as one SNP since they have the same genotypic frequencies for all breeds. When analyzed together in the association model, C41T and A468G SNPs were found to be confounding. When analyzed separately in the model, C41T and A468G were found to have similar output result, hence strongly suggesting they are one SNP. Results reported here are for C41T will also intuitively refer to A468G. The interaction between the marker and gender was tested, and it was found to be significant for WA and WW for both A11C and C1027T

and only with KPH for A11C. There was no interaction of any marker to the rest of the phenotypic traits. The interaction term was removed from the final model in those traits in which no gender by marker interaction was observed. But the interaction term was retained in traits in which gender by marker interaction was observed. The LSMEAN statement was used to separate means in those traits in which no gender by marker interaction was observed. The PROC GLM procedure was used to group and separate means by Duncan Multiple range test in which gender by marker interaction was observed.

Table 3.1. Primer sequences used in ADRB2 SNPs genotyping

SNP	Primers	Annealing temperature
A11C	CTGCGCGCCATGGG – Forward TCCAGCGTGACGTTTTGGT - Reverse	59.3^{o}C
C41T	GGAACCGCAGCGTCTTTT - Forward TCCAGCGTGACGTTTTGGT - Reverse	60.2^{o}C
A-408C	CGACCCGCCCTCCTAGA – Forward GCCCCTTCCCTCCCTTCT - Reverse	61.1^{o}C
A468G	TGCCTGCTGACCAAGAATAAGG – Forward AGAAGGAGGTAAGGCCAGACA - Reverse	61.1^{o}C
C1027T	GATTGCCTTCCAGGAGCTTCT – Forward GCCATTCCCATAGGCCTTCAAT - Reverse	61.1^{o}C

Figure 3.1. Allelic discrimination of the first 380 samples for C41T SNP of the ADRB2 gene

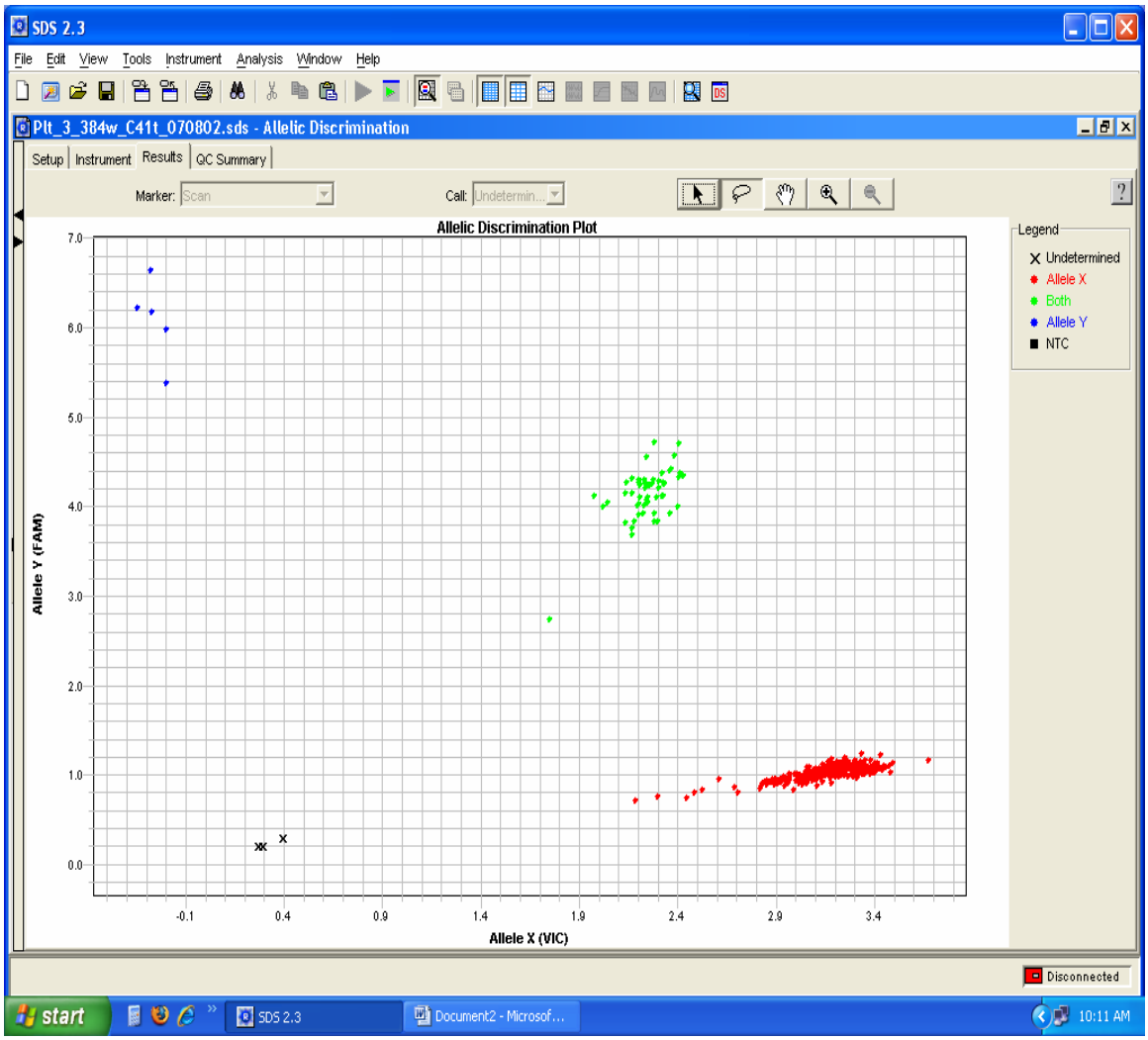
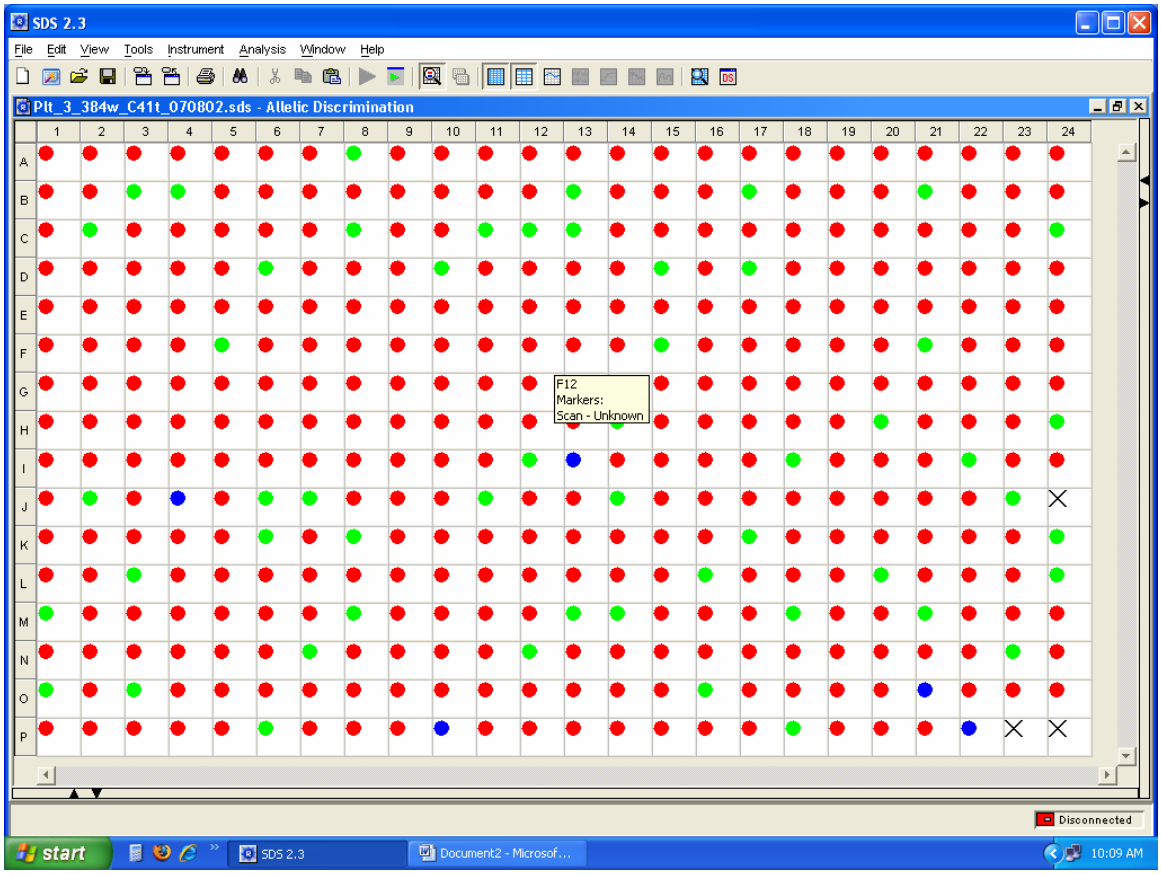


Figure 3.2. Appearance of the first 380 samples for discriminated alleles of C41T SNP on a 384 well plate



CHAPTER 4

RESULTS AND DISCUSSION

A total of 693 calves were evaluated for growth, carcass and meat characteristics at V bar V ranch. The phenotypic traits studied include BW, WA, WW, HCW, MS, FT, LM, KPH and YG. These calves were also genotyped for five SNPs (A11C, C41T, A-408C, A468G and C1027T) in the ADRB2 gene which were then associated with phenotypic traits studied. Results presented here are discussed under two categories i.e. description of the study population and the association of ADRB2 SNPs to phenotypic traits.

Description of the study population

Figure 4.1 shows the distribution of different breeds in a study population. A total of 693 calves were studied. These calves belonged to six breed categories namely Angus cross (4.33%), Red Angus cross (5.34%), Beefmaster cross (2.45%), Purebred Hereford (26.55%), Hereford cross (58.58%) and Waguli cross (2.74%). These are the animals which their phenotypic traits have been recorded and kept from birth to harvest at slaughter. They also had blood samples collected and kept for DNA extraction and genotyping. Purebred Hereford and Hereford cross formed the largest numbers of the study population; the Angus and Angus Red Cross were at the middle whereas the Waguli formed the smallest percent of the study population. This is due in part to the fact that Waguli breed was not introduced into the V-V population until 2003. It is clear from the distribution of breeds that Purebred Herefords and Hereford crosses make up the largest component, (85%) of the population.

The different breed groups in a study population were then subdivided according to gender (heifer or steer). Figure 4.2 shows the percentage distribution of gender of calves across breeds within the sample population. As expected, steers presented with the highest gender category in the study population. A total of 590 steers were recorded with 26 steers (3.75%) in the Angus cross, 33 steers (4.76%) in the Red Angus cross, 10 steers (1.44%) in the Beefmaster cross, 153 steers (22.08%) in the Purebred Hereford, 349 steers (50.36%) in the Hereford cross and 19 steers (2.74%) in the Waguli cross. A total of 103 heifers were recorded in the study population and formed the second largest gender category after steers. The Angus cross recorded 4 heifers (0.58%), the Red Angus cross had 4 heifers (0.58%), the Beefmaster had 7 heifers (1.01%), Purebred Hereford had 31 heifers (4.47%), Hereford cross had 57 heifers (8.23%) and the Waguli cross recorded with no heifers. Most of the heifers and the steers were observed both in the Purebred Hereford and Hereford cross breed that formed the largest sample size in the study population.

Figure 4.3 shows the distribution of BW in pounds across breed types in the sample population. There was a difference in birth weights (P=0.1) with purebred Hereford and Hereford cross calves having the highest birth weight relative to the rest of the breed types. Purebred Hereford calves had lower BW compared to Hereford cross calves.

Figure 4.4 shows the distribution of WA in days across breed types in the study population. Purebred Hereford and Hereford cross breed did not differ from each other on WA, but they differed from the rest of the breeds (P=0.05) by having less days to

weaning. Angus cross, Red Angus cross, Beefmaster cross and Waguli did not differ from each other on WA.

Figure 4.5 shows the distribution of WW in pounds across breed types. Purebred Hereford had lower WW (P=0.01) when compared to Hereford cross breed, Waguli cross and Angus cross calves. Angus cross, Purebred Hereford and the Waguli cross calves did not differ from each other, the same applies for Red Angus cross, Beefmaster and Purebred Hereford calves that did not differ from each other. Purebred Herefords and Hereford crosses had higher BW and weaned at earlier ages than the other four breeds. Purebred Herefords had lower WW than Hereford crosses demonstrating the well known effect of hybrid vigor. It is possible that the inherent capacity for and efficiency of protein deposition may contribute to these effects (Carr et al., 1977) seen in the purebred Hereford and Hereford cross breeds. These breeds in the feedlot have higher FT, low LM and grade relatively higher on YG than other breeds.

Figure 4.6 shows the HCW at harvest across breed types. Differences (P=0.1) were observed between Purebred Hereford and Red Angus cross. There were no differences for Purebred Hereford and Hereford cross animals on HCW compared to the rest of the breed types with exception of the red Angus cross that differed from Purebred Hereford. Lack of statistical differences between Purebred Hereford and Hereford cross, and with other breeds may be due to in part to a higher number of animals in these two breeds compared to the other four breeds that had smaller sample sizes. However, there appears to be lower overall carcass yield from Purebred Hereford.

Figure 4.7 shows the distribution of MS across breed types during harvest. Purebred Hereford numerically differed (P=0.1) from the other five breeds on MS. Purebred Hereford also presented with numerically higher MS (P=0.1) than Hereford cross. The remaining five breeds with exception of the Purebred Hereford, did not differ from each other on MS. As a function to determine quality grade, MS is subject to variation in fat distribution from one breed to the other and even so from one animal to the other. Practically it is important to note that, intramuscular fat deposition is influenced by an array of factors, including nutrition, and it occurs at a slower rate than internal and external fat deposition (Sainz and Hasting, 2002). Given this knowledge, it can be speculated that, the study breeds other than the Angus cross with relatively higher MS, may require a greater amount of time on finishing diets to allow for maximum rate of lean tissue gain and intramuscular fat development. It is of interest that the lowest numerical score was the Waguli cross which might have been predicted to have the highest MS.

Figure 4.8 shows the distribution of FT in inches across different breed types at harvest. The Waguli cross presented with lower FT (P<0.1) than Hereford cross, Purebred Hereford and Beefmaster cross. Waguli cross did not differ from Angus cross and Red Angus cross on FT. Purebred Hereford presented with higher FT than the Hereford cross but did not differ from the Beefmaster cross (P<0.1).

Figure 4.9 shows the distribution of LM across breed types at harvest. Pure Hereford had significantly lower LM (P<0.1) than Hereford cross, Waguli cross, Angus cross and Red Angus Cross but did not differ from the Beefmaster cross. The Angus cross, Red Angus

cross and the Waguli cross presented with higher LM but they were not different from each other.

Figure 4.10 shows the distribution of KPH across different breed types at harvest. Purebred Hereford had significantly lower KPH ($P<0.1$) than the Angus cross, Red Angus cross, Beefmaster cross and Purebred Hereford but did not differ from the Waguli cross.

Figure 4.11 shows the distribution of YG across breeds during harvest. The Waguli cross breed presented with significantly lower YG ($P<0.1$) than Beefmaster cross, Purebred Hereford and Hereford cross breed. Hereford cross had significantly lower YG than Purebred Hereford. The Angus cross, Red Angus cross and Beefmaster cross did not differ from each other on YG.

Generally, feedlot operators attempt to time the slaughter of commercial cattle at the point where the ratio of lean to fat carcass composition result in high probability of optimal grades (Kononoff et al., 2005). But as mentioned earlier, the weight at which cattle reach the same chemical composition is influenced by a number of factors including plane of nutrition. Thus rearing all these six breeds under the same management will result in variation in body composition hence, carcass grade. For example, it is not surprising that five breeds studied other than Waguli cross fatten more rapidly, hence acquire more body fat than they can make lean mass. This makes them acquire more carcass fat levels with a net effect of low probability of grading with high YG.

Description of the ADRB2 SNPs association analyses

Table 4.1 shows the effect of gender on phenotypic characteristics studied. Gender had no effect (P>0.1) on WA, FT, LM and YG but had effect on the rest of the phenotypic traits studied. As mentioned earlier in the review, the ADRB2 gene plays a role in partitioning nutrients between lean and fat in beef cattle approaching market weight. It is therefore not surprising when the SNP association model cannot pick significant interaction of gender with ADRB2 gene markers to FT, LM, and therefore YG.

Table 4.2 shows the genotype frequencies for the ADRB2 gene SNPs studied. Higher genotypic frequencies are observed between Purebred Hereford and Hereford cross breeds which form the largest percentage of the study population. SNP C41T and A468G presented with the same frequencies in the opposite direction as far as allele substitution at their respective loci is concerned. When the two SNPs were run in the association model, they were found to confound each other, and when analyzed separately, they presented with similar results. Therefore C41T results described here will also refer to similar results with A468G. A-408C showed extreme genotypic frequency being almost 100% homozygous for CC, indicating complete substitution of A allele in the study population at this locus with no homozygous individuals for AA genotype or heterozygous individuals for AC genotype. The 100% presence of CC genotype at the A-408C makes it uninformative in this study population. However, in the Monsanto company study that involved association of milk traits to this marker, this was found to be the marker that had broadest range of marker trait associations (Du et al.,). With this one genotype level, the A-408C SNP was not analyzed in the association study since it

presented itself with no degrees of freedom. On the other hand, some genotypes for all these SNPs were completely absent in some of the breeds. For example, at A11C locus, no AA genotype was observed in all the study breeds. Similarly at C41T locus, no CT genotype was observed in the Beefmaster cross and no TT genotype was observed in the Beefmaster cross. At A-408C neither either AA genotype nor AC genotype was observed in all breeds studied. At A468G no AA genotype was observed in the Beefmaster cross and no AG genotype was observed in the Waguli cross. At the C1027T locus, no CT genotype was observed in the Red Angus cross, Beefmaster cross and Purebred Hereford whereas no TT genotype was observed in all breeds studied. Some of the observed genotypes were present in low frequency in all the five loci studied. Both the absence and low frequency of these genotypes interferes with accurate assessment of their association to growth, carcass and meat characteristics, especially if any of these effects have a dominant or recessive mode of action.

Four of these SNPs (A11C, C41T, A468G and C1027T) exists within the coding region of the gene, and change the amino acid sequence of the protein. These potentially change the structure, and subsequently affect the ability of the receptor protein to discharge its proper cellular function (thus leading to a phenotypic effect). For example, for the C41T locus, the substitution of T (thymidine) for C (cytosine) at position 41 of the ADRB2 gene alters the amino acid sequence of the ADRB2 protein by replacing a proline with a leucine amino acid residue at position 14 of the protein. At the A468G locus, substitution of A (adenine) for G (guanidine) at position 468 of the ADRB2 gene replaces methionine residue with an isoleucine residue at amino acid position 156 of the ADRB2 protein.

Table 4.3 shows the least significance differences for the association of SNPs in the ADRB2 gene with growth, carcass and meat characteristics in beef cattle. Results for C41T will also refer to A468G since they are the same SNPs. The interaction effect was observed between A11C and gender for WA (P=0.0359), A11C and gender for KPH (P=0.0513). There was also an interaction effect between C1027T and gender for KPH (P=0.0178).

When the Chi-square test was performed to determine the effect of genotype on breed, only C41T/A468G locus had effect (P=0.0232). As discussed earlier C41T and A468G loci are located in the coding region of the ADRB2 gene and they are associated with amino acid change following nucleotide substitution that changes the protein structure and therefore function (Du et al.,). Therefore it is likely that the effects of breeds seen on various traits discussed above in the V bar V ranch are mediated through the C41T and A468G loci and not otherwise. Du et al., when studying these loci in dairy cattle, found that, in addition to C41T and A468G loci, also A11C and more so A-408C had effect on milk traits. In V bar V ranch A-408C locus appear to be uninformative since it is associated with 100% homozygozity for CC genotype.

Table 4.4 shows the interaction effect of A11C and C1027T on gender of calves for WA and KPH. Steers with complete substitution of C with A allele forming a CC genotype in the A11C marker wean at higher age than heifers. Being heterozygous for AC genotype at the A11C locus, both heifers and steers wean at higher age compared with both heifers ands steers which are homozygous for the CC genotype in the A11C locus. However, heifers which are homozygous for CC genotypes at the A11C locus wean at earlier age

compared to both steers in the same category and being heterozygous at A11C locus. Steers which are homozygous at the A11C locus have lower KPH compared to heifers. Heifers have significantly (P<0.0001) lower mean BW compared steers (Table 4.1), and therefore wean at earlier age when compared to steers. As discussed before this inherent lower BW in heifers (Carr et al., 1977) is likely to affect their WA when compared to steers. In addition complete presence of the CC genotype at A11C in steers likely leads to amino acid sequence change of the ADRB2 protein that is associated more with lower KPH at higher WA than in heifers. For C1027T locus, steers which are homozygous for the unsubstituted genotype CC wean at higher age compared to heifers. Results for heterozygotes for CT genotype in the C1027T are difficult to explain due to limited number of animals. However, it appears that being heterozygous for CT genotype at C1027T locus, steers wean at earlier age. So it is probable that A11C and C1027T loci in the coding region of the ADRB2 protein influence WA in these calves.

Tables 4.5 and 4.6 show the association of the A11C, C41T/A468G and C1027T for the traits where the interaction was not observed. For all these traits, no association with the SNP was observed (Table 4.3). Even when the interaction term was removed and means separated by LSMEANS statement, only significant differences were observed for the BW (P=0.0625) for C41T, HCW (P=0.0873) for A11C and WW (P=0.0397) for C1027T. As discussed earlier, the C41T locus was effected by breed and descriptive statistics showed differences for BW between the purebred Hereford and Hereford cross. These are breeds that had significantly higher BW and wean at significantly early age. In these breeds, the CC genotype substitution for A allele at the A11C locus accounts for 91.85%

and 91.38% of the genotypic frequencies respectively. It is likely that this change in nucleotide has effect on the ability of these animals right at birth to put up more muscle mass and therefore wean earlier.

Gender also has significantly higher effect on BW (P<.0001) with steers having higher BW than heifers. Subsequently steers with CC genotype at A11C locus wean at significantly higher age than heifers. Steers with unsubstituted CC genotype at C1027T locus wean at higher age than heifers. Having both alleles, i.e AC for A11C locus is associated with lower age at weaning for steers than heifers. It is likely that for this allele more muscle weight hence early WA is not related to allele change at this locus. Steers that are heterozygous at C1027T wean at lower weights than being homozygous for the two alleles at this locus. At the A11C locus, heifers in both heterozygous form and homozygous form for allele substitution have lower WW compared to steers. Steers presents with higher WW in heterozygous form at the A11C locus. Therefore A11C locus in the ADRB2 gene presents the best alternative marker for breeders and managers at V bar V ranch that will in the future consider selecting steers that right at birth will be associated with higher WW at early age.

Figure 4.1. Percentage distribution of Breeds within the Sample Population

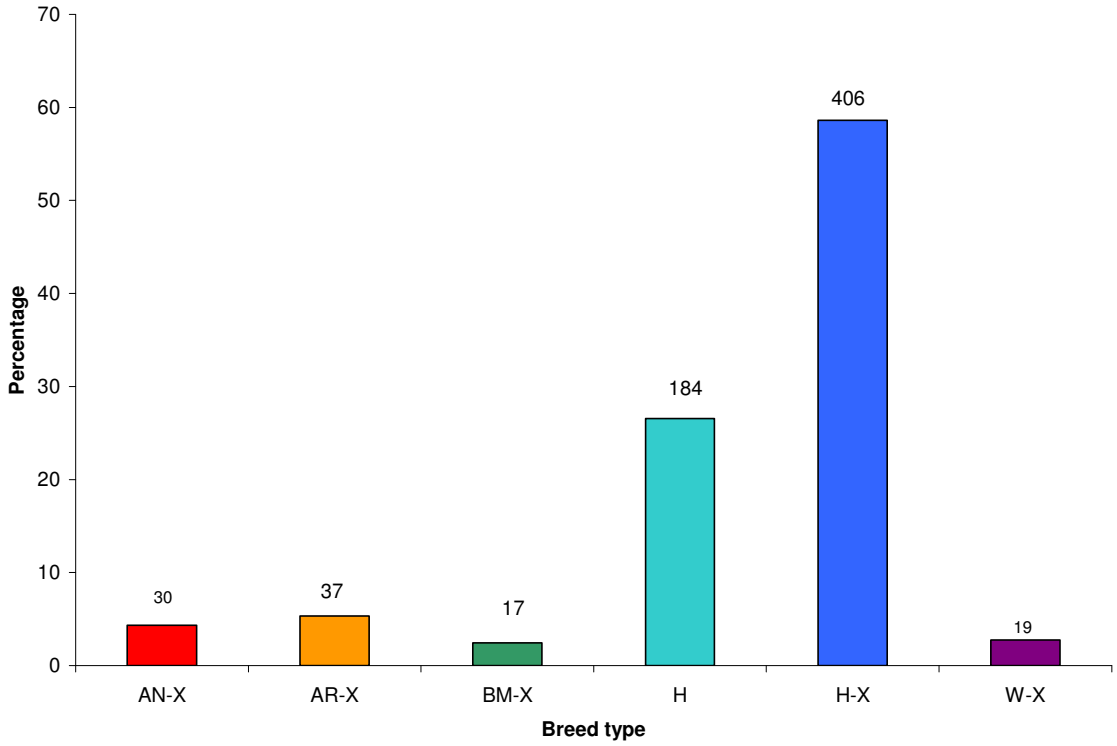


Figure 4.2. Percentage distribution of Gender of Calves across Breeds within the Sample Population

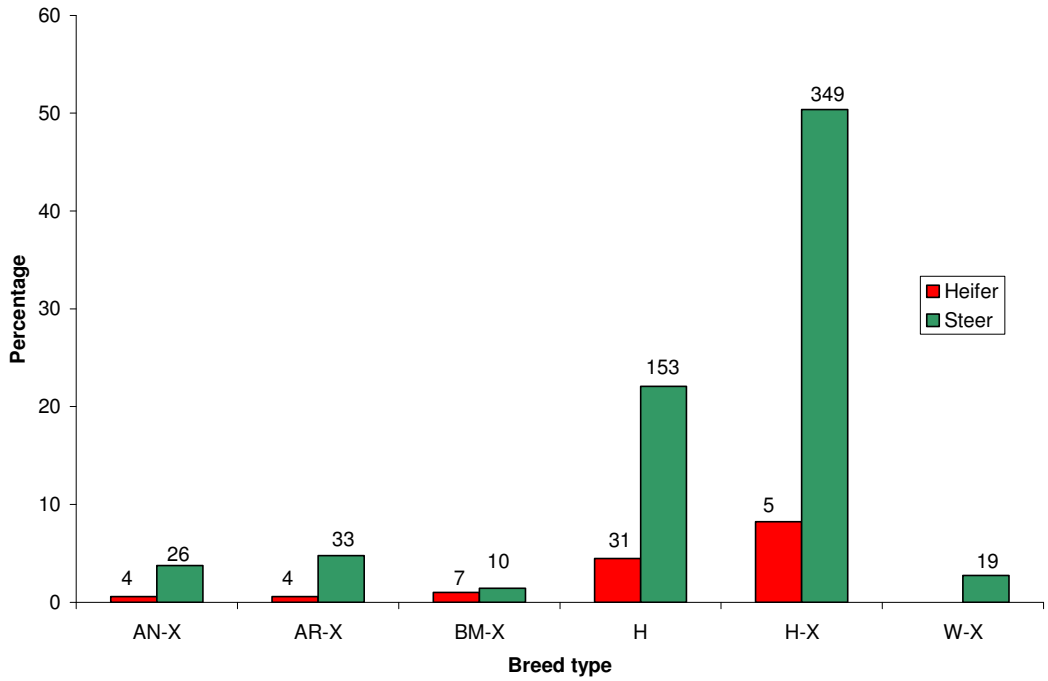
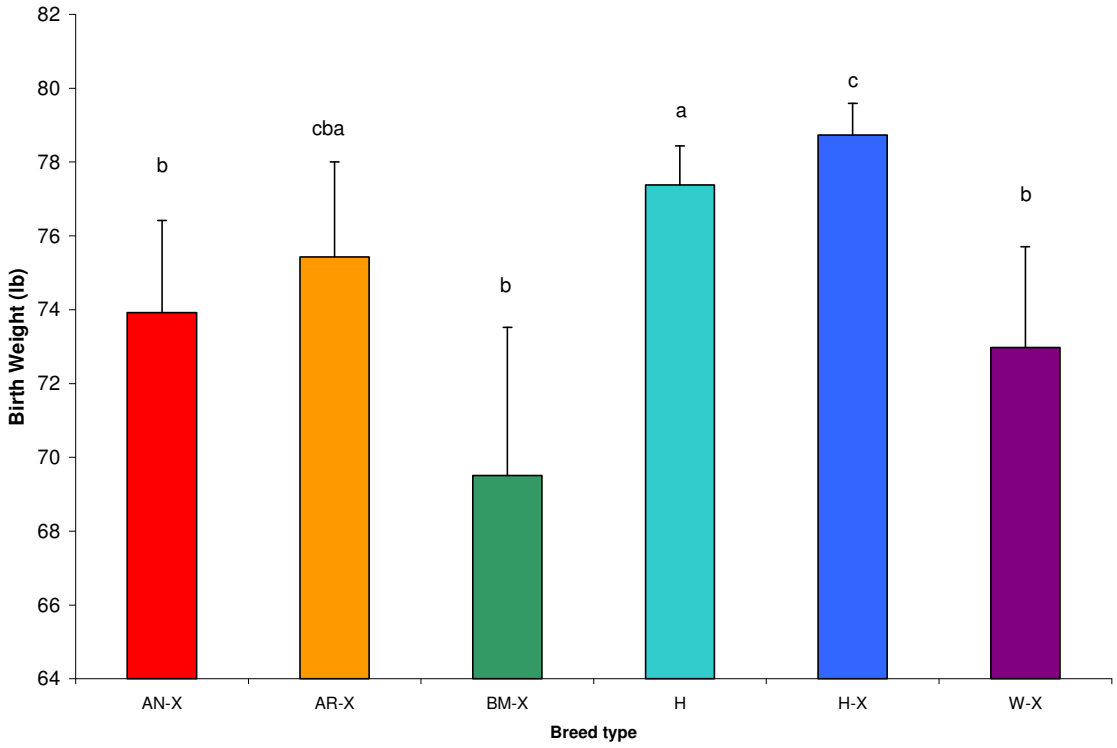
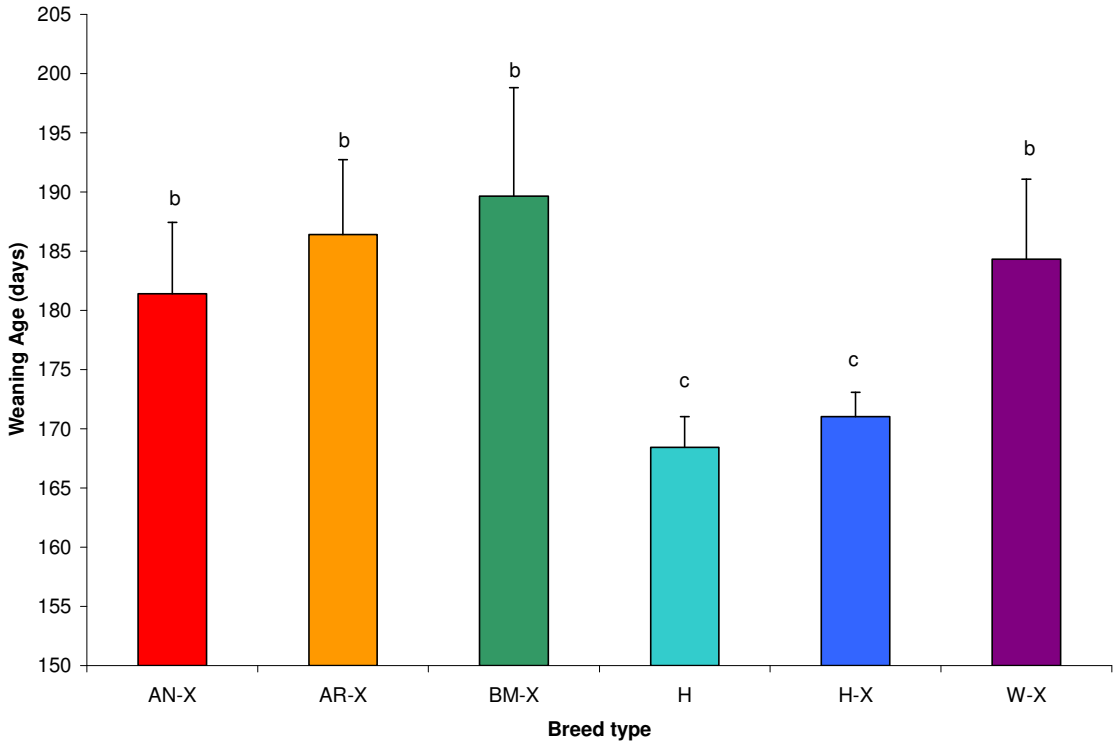


Figure 4.3. Effect of Breed on BW in Beef Calves



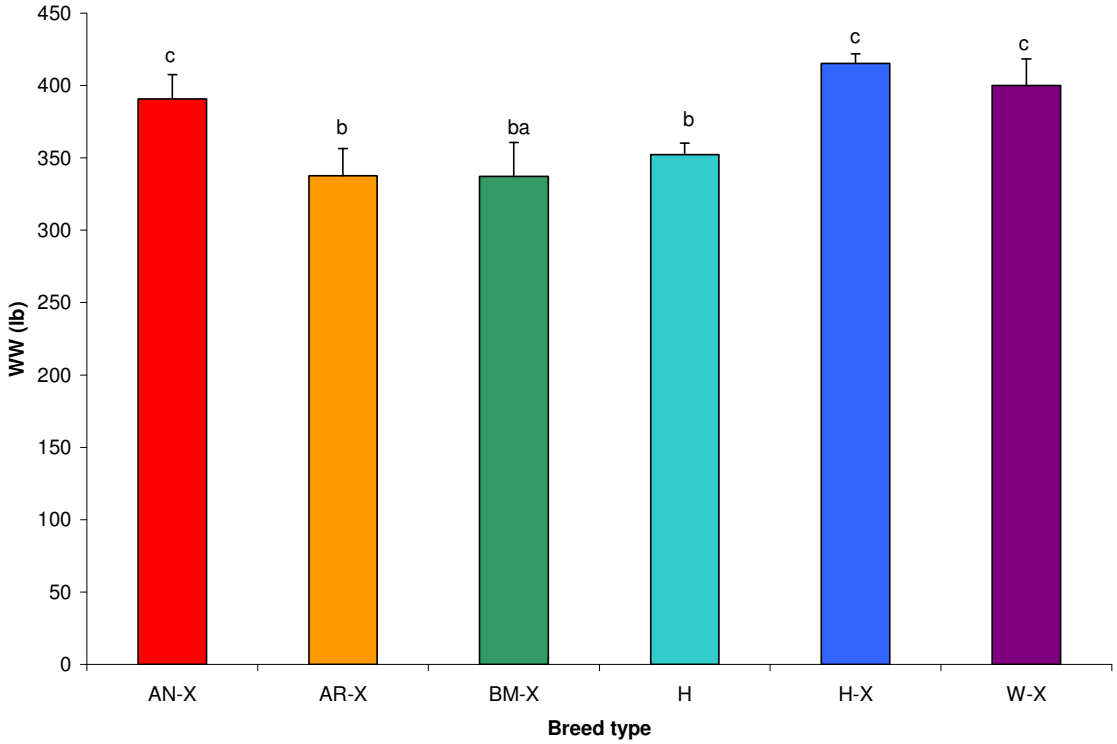
[a,b,c]Least square means in a bar column with different letters were significantly different (P=0.1)

Figure 4.4. Effect of Breed on WA in Beef Calves



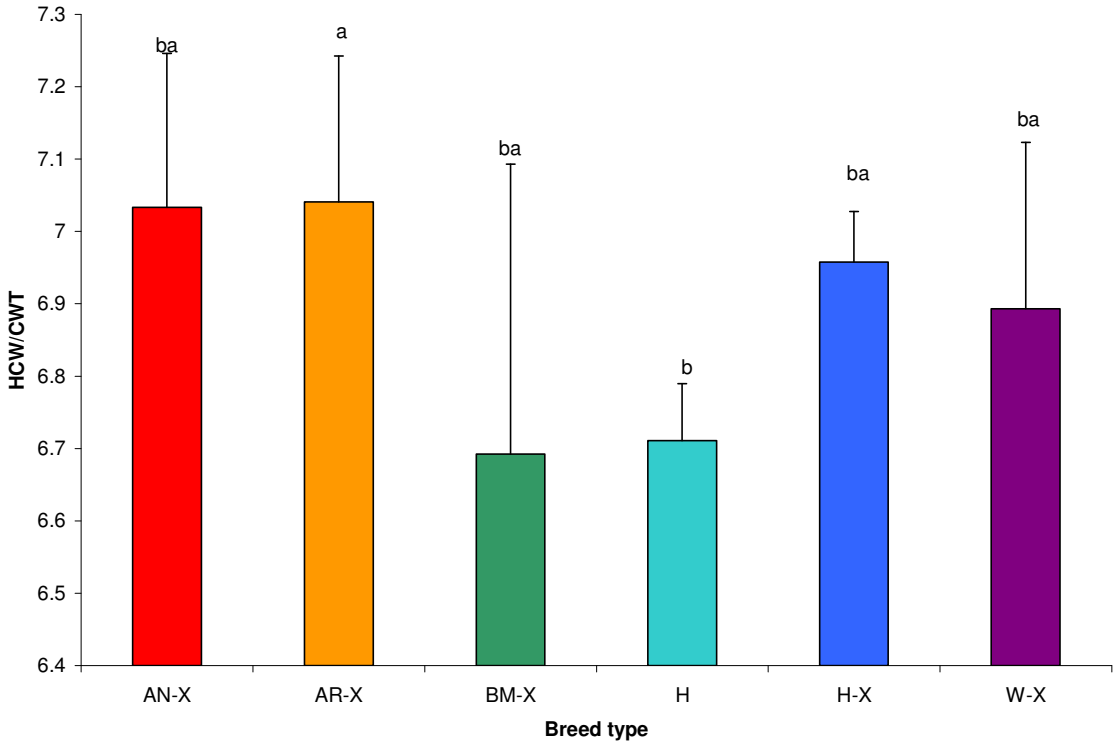
[a,b,c]Least square means in a bar column with different letters were significantly different (P=0.05)

Figure 4.5. Effect of Breed on WW in Beef Calves



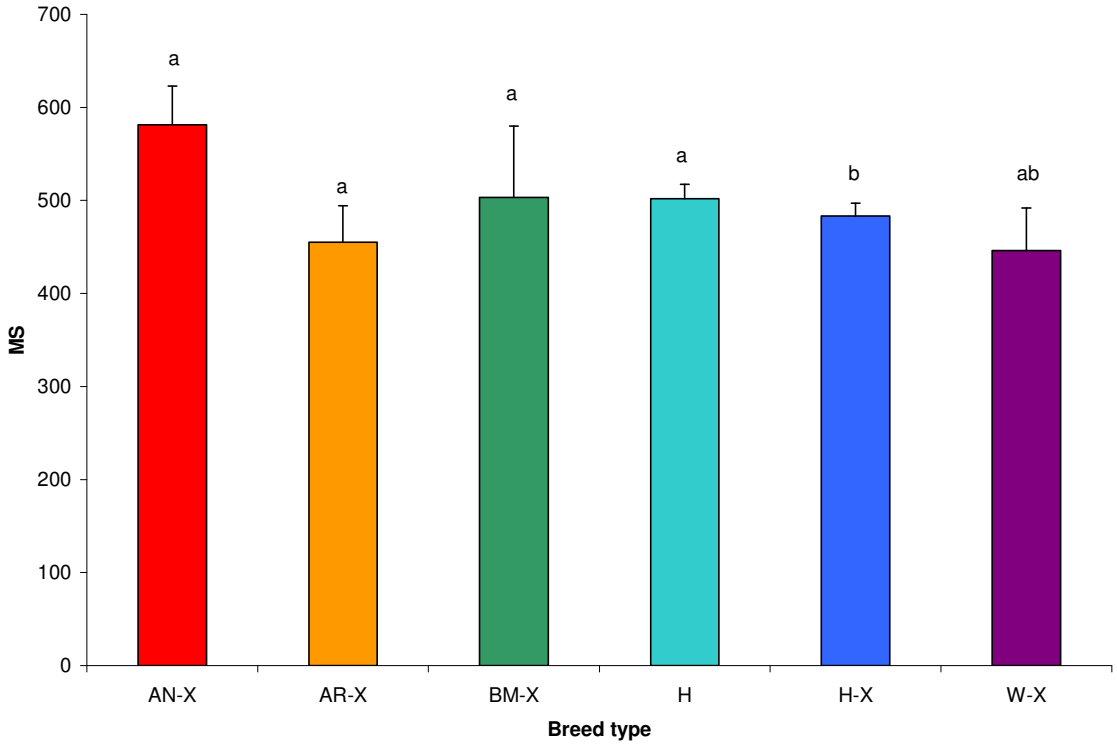
[a,b,c]Least square means in a bar column with different letters were significantly different (P=0.01)

Figure 4.6. Effect of Breed on HCW/CWT during Harvest



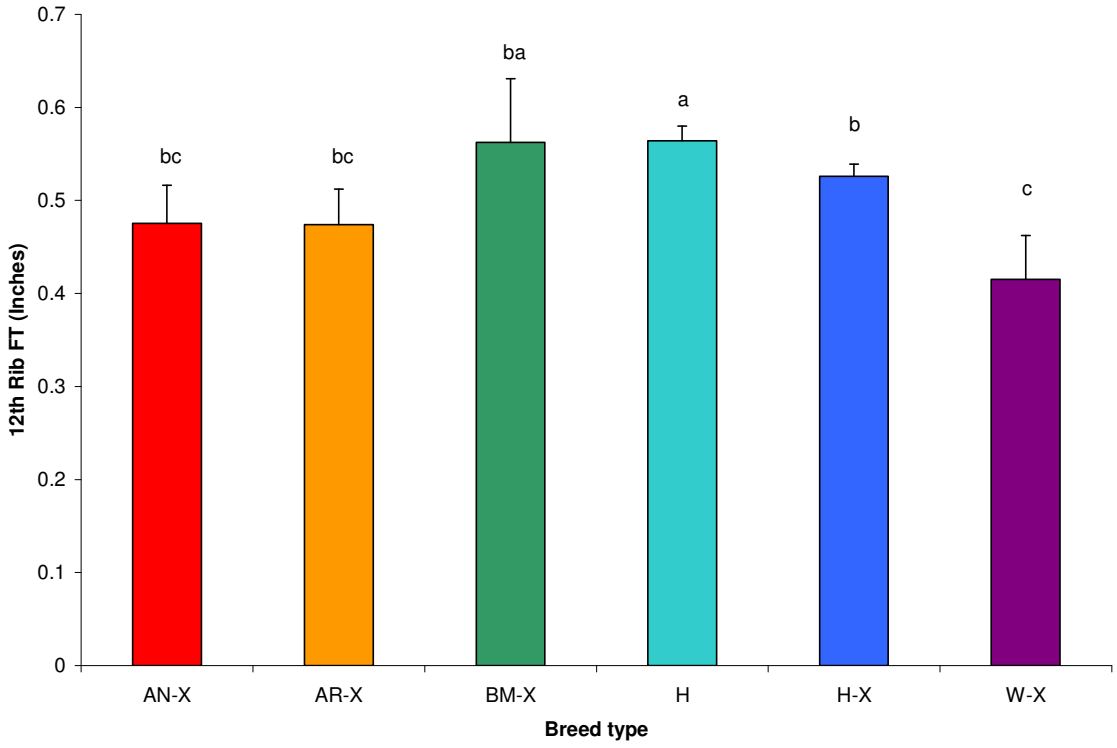
[a,b]Least square means in a bar column with different letters were significantly different (P=0.1)

Figure 4.7. Effect of Breed on MS during Harvest



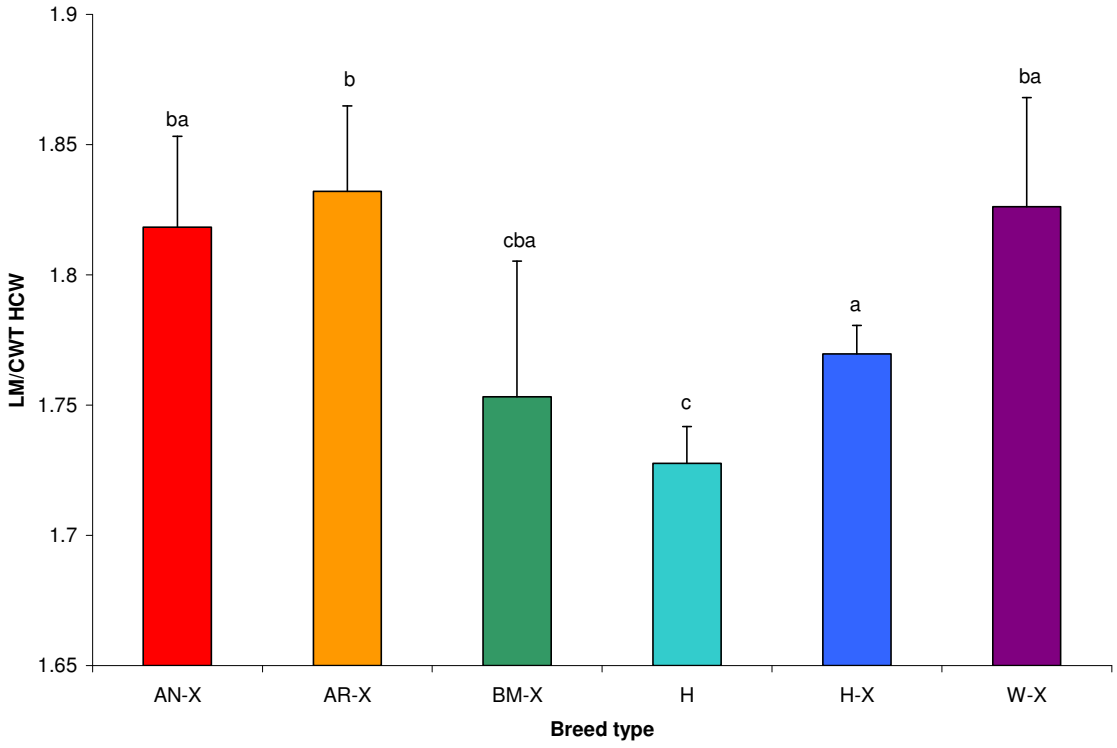
[a,b]Least square means in a bar column with different letters were significantly different (P=0.1)

Figure 4.8. Effect of Breed on FT during Harvest



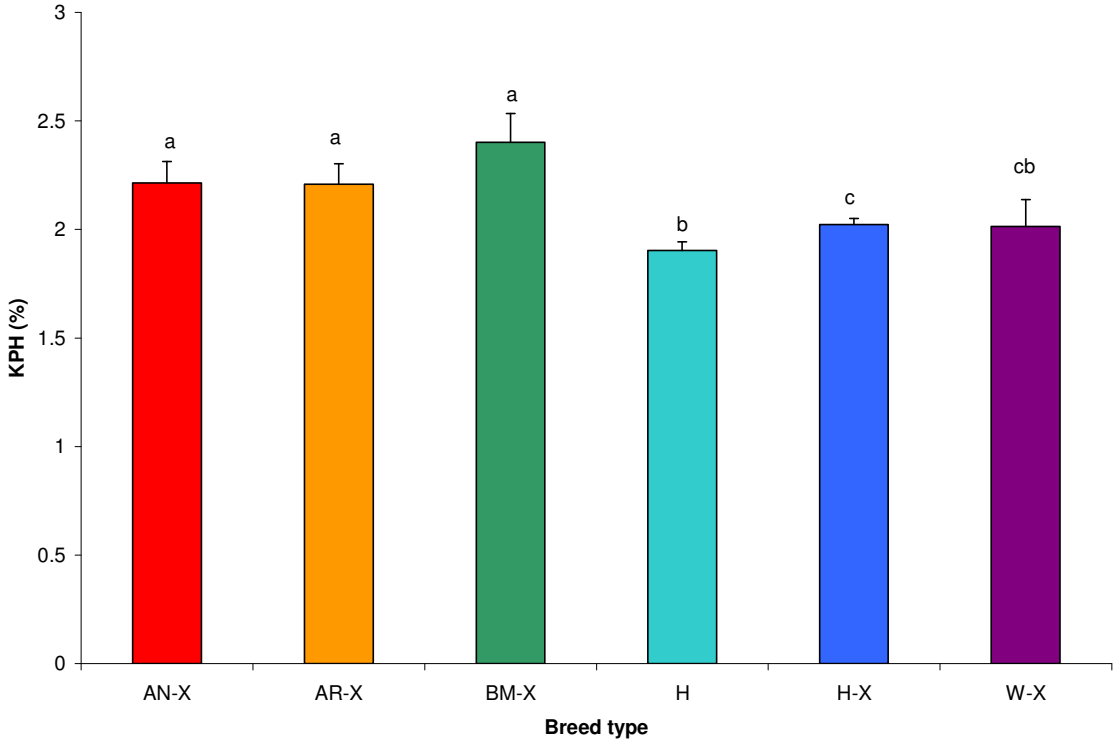
[a,b,c]Least square means in a bar column with different letters were significantly different (P<0.1)

Figure 4.9. Effect of Breed on LM/CWT HCW during Harvest



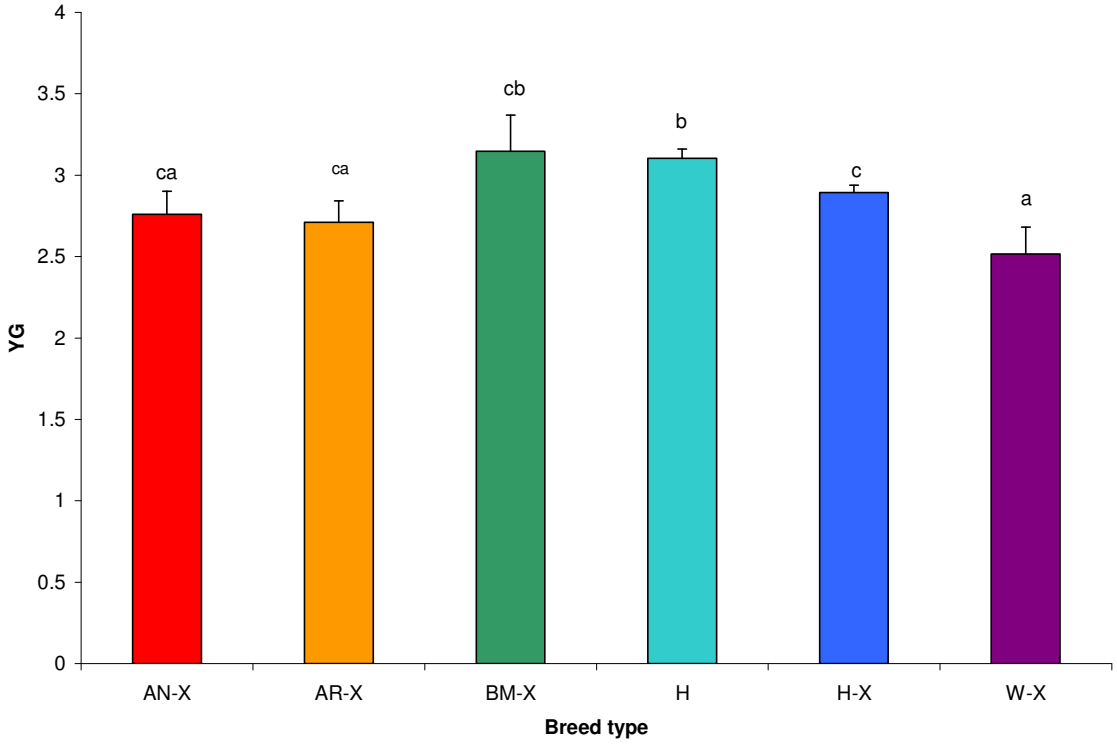
[a,b,c]Least square means in a bar column with different letters were significantly different (P<0.1)

Figure 4.10. Effect of Breed on KPH during Harvest



[a,b,c]Least square means in a bar column with different letters were significantly different (P<0.1)

Figure 4.11. Effect of Breed on YG during Harvest



[a,b,c]Least square means in a bar column with different letters were significantly different (P<0.1)

Table 4.1. Effect of Gender on growth, carcass and meat characteristics

	Gender		
Trait/item	Heifer	Steer	Gender effect
BW	70.89(1.08)[b]	79.26(0.81)[a]	P<0.0001
WA	170.40(2.93)[b]	173.72(2.12)[b]	P>0.1
WW	372.86(8.91)[b]	412.03(6.80)[a]	P<0.0001
HCW	662(0.08)[b]	694(0.05)[a]	P<0.0001
MS	514.91(17.17)[b]	487.92(11.76)[a]	P<0.1
FT	0.54(0.02)[b]	0.52(0.01)[b]	P>0.1
LM	12.12(0.16)[b]	12.21(0.11)[b]	P>0.1
LM/CWT HCW	1.82(0.02)[b]	1.76(0.05)[a]	P<0.001
KPH	2.23(0.05)[b]	1.99(0.02)[a]	P<0.0001
YG	2.93(0.07)[b]	2.91(0.04)[b]	P>0.1

[a,b]Row least square means with different superscript differ

Table 4.2. Percentage distribution of genotypes from different ADRB2 SNPs across the study population

		Breed type						
SNP	Genotype	AN-X	AR-X	BM-X	H	H-X	W-X	Total
A11C	AA%	0	0	0	0	0	0	0
	AC%	10	8.11	5.88	6.52	8.37	0	7.65
	CC%	90	91.89	88.24	91.85	91.38	100	91.63
	UN%	0	0	5.88	1.63	0.25	0	0.72
	No	30	37	17	184	406	19	693
C41T	CC%	76.67	86.49	76.47	72.28	83.00	100	80.37
	CT%	20.00	8.11	23.53	26.09	16.26	0	18.33
	TT%	3.33	2.70	0	1.63	0.49	0	1.01
	UN%	0	2.70	0	0	0.25	0	0.29
	No	30	37	17	184	406	19	693
A-408C	AA%	0	0	0	0	0	0	0
	AC%	0	0	0	0	0	0	0
	CC%	100	100	100	99.46	99.26	100	99.42
	UN%	0	0	0	0.54	0.74	100	0.58
	No	30	37	17	184	406	19	693
A468G	AA%	3.33	2.70	0	1.63	0.49	0	1.01
	AG%	20.00	8.11	23.53	25.00	16.50	0	18.18
	GG	76.67	89.19	76.47	72.28	82.51	100	80.23
	UN%	0	0	0	1.09	0.49	0	0.58
	No	30	37	17	184	406	19	693
C1027T	CC%	100	97.30	100	99.46	98.78	100	98.99
	CT%	0	2.77	0	0	0.98	0	0.72
	TT%	0	0	0	0	0	0	0
	UN%	0	0	0	0.54	0.24	0	0.29
	No	30	37	17	184	406	19	693

Table 4.3. Least significance differences for the association of SNPs in the ADRB2 gene with growth, carcass and meat characteristics in the beef cattle population

	SNP in the ADRB2 gene[1]					
Trait	A11C	C41T/A468G	C1027T	A11CxGender	C41TxGender	C1027TxGender
BW	0.9038	0.2602	0.1285			
WA		0.3406		0.0359		0.0178
WW		0.3584		0.1075		0.0663
HCW	0.4425	0.6287	0.9662			
MS	0.9569	0.8828	0.8168			
FT	0.9265	0.7841	0.3840			
LM	0.4213	0.2717	0.6257			
KPH		0.1381	0.5408	0.0513		
YG	0.5817	0.9897	07749			
P-Value for SNP effect on breed[2]						
	0.7870	0.0232	0.5174			

[1]P-values for least significant differences observed for study traits at different loci in the ADRB2 gene

[2]Effect of genotype on breed obtained by Chi square analysis

Table 4.4. Association of SNPs in the ADRB2 gene with WA, WW and KPH in the study beef cattle population

	Trait[1]								
Item	WA	SD	No	WW	SD	No	KPH	SD	No
A11CxGender									
AC Heifer	196.00	32.02	8	415.25	72.86	8	2.00	0.60	8
AC Steer	178.65	28.68	43	428.86	61.87	43	2.09	0.61	42
CC Heifer	166.18	29.46	92	353.12	96.30	92	2.25	0.63	90
CC Steer	174.63	27.14	536	420.74	83.72	536	1.97	0.49	527
C1027TxGender									
CC Heifer	168.25	30.59	99	357.05	95.76	99			
CC Steer	175.06	27.27	575	422.02	82.06	575			
CT Heifer	200	-	1	461.00	-	1			
CT Steer	156.25	17.59	4	323.75	57.02	4			
P-Value for interaction term and fixed effects									
A11CxGender	0.0844			0.1614			0.5704		
C1027TxGender	0.0965			0.0019					
Breed	0.1625			0.0094			0.5150		
Gender	0.3082			0.4844			0.5449		

[1]Traits in which SNP by gender interaction was observed
[2]Duncan means for which A11C by gender interaction was observed
[3]Duncan means for which C1027T by gender interaction was observed
[4]Values obtained by Duncan multiple range test following SNP by gender interaction

Table 4.5. Association of SNPs in the ADRB2 gene with BW, HCW and MS in the study beef cattle population

		Trait					
Item		BW	SEM[1]	HCW	SEM[1]	MS	SEM[1]
SNP lsmeans[2]							
A11C	AC	73.51[b]	2.72	6.62[b]	0.21	544.90	43.28[b]
	CC	73.13[b]	2.42	6.78[a]	0.18	545.77	38.54[b]
C41T	CC	70.92[a]	2.24	6.68[b]	0.17	534.14	35.64[b]
	CT	72.69[b]	2.42	6.63[b]	0.18	544.27	38.38[b]
	TT	76.36[b]	3.89	6.79[b]	0.29	557.60	60.88[b]
C1027T	CC	74.78[b]	1.69	6.74[b]	0.13	512.93	26.87[b]
	CT	71.87[b]	4.08	6.66[b]	0.31	577.74	64.75[b]
Breed lsmeans[3]							
AN-X		72.63[b]	3.26	6.87[b]	0.27	628.91	54.31[b]
AR-X		73.75[b]	3.23	6.82[ba]	0.25	503.60	51.16[b]
BM-X		69.80[c]	4.46	6.56[ba]	0.42	550.76	83.65[b]
H		76.21[ba]	2.49	6.53[a]	0.19	553.23	38.96[b]
H-X		76.88[b]	2.39	6.76[b]	0.18	534.46	37.84[b]
W-X		70.64[c]	3.50	6.65[ba]	0.28	501.07	58.74[b]
Gender lsmeans[4]							
Heifer		69.18[b]	2.59	6.55[b]	0.20	556.91	41.30[b]
Steer		77.47[a]	2.51	6.85[a]	0.19	533.77	39.55[a]
P-Value for SNP and fixed effects[5]							
A11C		0.7687		0.0873		0.9648	
C41T		0.0625		0.6137		0.7378	
C1027T		0.4357		0.7890		0.2751	
Breed		0.0635		0.0057		0.1372	
Gender		P<0.0001		P<0.0001		0.1160	

[1]Standard error of the mean for a given specific trait

[2]Least square means for SNP genotypes obtained by Least Square Significance Difference (LSD) following absence of SNP by gender interaction

[3]Breed Least square means obtained by LSD following absence of SNP by gender interaction

[4]Gender Least square means obtained by LSD following absence of SNP by gender interaction

[5]Values obtained by LSD following absence of SNP by gender interaction

[a,b]No SNP by trait interaction occurred(P>0.1). Therefore main effect trait characteristics were analyzed. Row means with different superscript differ

Table 4.6. Association of SNPs in the ADRB2 gene FT, LM and YG in the study beef cattle population

		Trait					
Item		FT	SEM[1]	LM	SEM[1]	YG	SEM[1]
SNP lsmeans[2]							
A11C	AC	0.53[b]	0.05	1.83[b]	0.05	2.89[b]	0.19
	CC	0.53[b]	0.04	1.82[b]	0.04	2.86[b]	0.17
C41T	CC	0.53[b]	0.04	1.80[b]	0.04	2.89[b]	0.15
	CT	0.51[b]	0.04	1.81[b]	0.04	2.88[b]	0.16
	TT	0.55[b]	0.07	1.87[b]	0.07	2.86[b]	0.27
C1027T	CC	0.51[b]	0.03	1.84[b]	0.03	2.85[b]	0.11
	CT	0.55[b]	0.08	1.81[b]	0.08	2.90[b]	0.29
Breed lsmeans[3]							
AN-X		0.50[b]	0.06	1.85[b]	0.05	2.78[c]	0.21
AR-X		0.50[b]	0.06	1.87[b]	0.05	2.73[ca]	0.20
BM-X		0.58[ba]	0.08	1.78[ba]	0.07	3.15[ba]	0.28
H		0.59[a]	0.05	1.76[a]	0.04	3.14[b]	0.17
H-X		0.55[b]	0.04	1.81[c]	0.04	2.92[a]	0.17
W-X		0.44[cb]	0.07	1.88[b]	0.06	2.53[c]	0.24
Gender lsmeans[4]							
Heifer		0.54[b]	0.05	1.86[b]	0.05	2.87[b]	0.18
Steer		0.52[b]	0.05	1.79[a]	0.04	2.88[b]	0.17
P-Value for SNP and fixed effects[5]							
A11C		0.8951		0.9207		0.7737	
C41T		0.5324		0.3889		0.9789	
C1027T		0.6247		0.6636		0.8289	
Breed		0.0052		0.0009		<0.0001	
Gender		0.3508		0.0001		0.7949	

[1]Standard error of the mean for a given specific trait

[2]Least square means for SNP genotypes obtained by Least Square Significance Difference (LSD) following absence of SNP by gender interaction

[3]Breed Least square means obtained by LSD following absence of SNP by gender interaction

[4]Gender Least square means obtained by LSD following absence of SNP by gender interaction

[5]Values obtained by LSD following absence of SNP by gender interaction

[a,b]No SNP by trait interaction occurred($P>0.1$). Therefore main effect trait characteristics were analyzed. Row means with different superscript differ

CHAPTER 5

IMPLICATIONS

The results reported here do not support the use of previously identified markers in dairy cattle, in beef cattle population for phenotypic traits during harvest. Since KPH is subjectively estimated, so far no specific conclusion can be made on the effect of A11C marker on KPH. However, these markers appear to be of applicable use for growth traits (WA and WW). This does not necessarily indicate that variations in the ADRB2 gene identified previously in dairy cattle do not exists in this study beef population for traits at harvest, but suitable markers should be developed for this beef population including further exploration of markers that were not associated to milk traits in dairy cattle. A strong opportunity exists to associate ADRB2 SNPs to phenotypic traits. This is because at least one SNP in the ADRB2 gene had been reported to be associated to back fat (Schimpf et al., 2001) and that fairly strong heritability exists for phenotypic traits studied (Koch et al., 1982).

REFERENCES

1. Ahlquist, R.P. (1948). Study of adrenotropic receptors. American Journal of Physiology. 153:586-600
2. Arch, J.R.S and Kaumann, A.J. (1993). β_3 and atypical β-adrenoreceptors. Medical Research Review. 13;663-729
3. Beauchemin, V.r., Thomas, M.G., franke, D.E., and Silver, G.A., (2006). Evaluation of DNA polymorphisms involving growth hormone relative to growth and carcass characteristics in Brahaman Steers. Genetics and Molecular Research. 5(3): 438-447
4. Bell, A.W., Bauman. D.E., Beermann, D.H., and Harrell, R.J., (1998). Nutrition, Development and Efficacy of Growth Modifiers in Livestock species. American society for Nutritional Sciences Symposium: The Roles of Nutrition, Development and Hormone Sensitivity in the Regulation of Protein Metabolism.
5. Bergen, W.G., Johnson, S.e., Skjaerlund, D.M., Babiker, A.S., Ames, N.K., Merkel, R.A., and Anderson, D.B., (1989). Muscle protein metabolism in finishing pigs fed ractopamine. Journal of Animal Science. 67: 2255-2262
6. Blum, J.W., Schams, D., and Bruckmaier, R (1989). Catecholamines, oxytocin and milk removal in dairy cows. Journal of Dairy Research. 56: 167-177
7. Bohorov, O., Buttery, P.J., Corrcia, J.I.R.D., and Soar, J.B., (1987). The effect of β_2-adrenergic agonist clenbuterol or implantation with estradiol plus

trenbolone acetae on proteinmetabolism in wether lambs. British Journal of Nutrition. 57:99-107

8. Brunsch,C., Sternstein, I., Reinecke,P., and Bieniek, J., (20020. Analysis of associations of PIT1 genotypes with growth, meat qulity and carcass composition traits in pigs. Journal of Applied Genetics. 43(1):85-91

9. Brym, P., Kaminski, S., and Wojcik, E., (2005). Nucleotide sequence polymorphism within exon 4 of the bovine prolactin gene and its associations with milk performance traits. Journal of Applied Genetics. 45(2): 179-185

10. Buchanan, F.C., Fitzsimmons, C.J., Van Kessel, A.G., Thue, T.D and Schmutz, S.M., (2002). A missense mutation in the bovine leptin gene affects carcass fat content and mRNA levels. Genetics Selection Evolution. 34:105-106

11. Burson, D.E., (2004). Quality and yield grades for beef carcasses. North Central Regional Extension Publication No. 357. http://ianrpubs.unl.edu/beef/rp357.htm

12. Byrem, T.M., Beermann, D.H., and Robinson, T.F., (1998). The Beta Agonist cimaterol directly enhances chronic protein accretion in skeletal muscle. Journal of Animal Science. 76:988-998

13. Carr, J.R., Boorman, K.N., and Cole, D.J.A., (1977). Nitrogen retention in the pig. British Journal of Nutrition. 37:143-155.

14. Cheong, H.S., Yoon, D., Kim, L.H., Park, B.L., Choi, Y.H., Chung,E.R., Cho, Y.M., Park, E.W., Cheong, I., O, S., Yi, S., Park, T., and Shin, H.D (2006).

Growth hormone releasing hormone (GHRH) polymorphisms associated with carcass traits of meat in Korean cattle. BMC Genetics. 7:35 – 41

15. Civantos, C.B., and de Artinamo, A.A., (2001). Alpha adrenoreceptor subtypes. Pharmacological Research. 44: 195-208.

16. Custom TaqMan SNP Genotyping assays Protocol Applied Biosystems 06/2006 http://docs.appliedbiosystems.com/pebiodocs/04334431.pdf

17. Dawson, J.M., Buttery, P.j., Lammiman, M.J., Soar, J.B., Essex, C.P., Gill, M., and Beever, D.E (1991). Nutritional and Endocrinological manipulation of lean deposition in forage fed steers. British Journal of Nutrition. 66: 171-185.

18. Du, F., Grosz, M., Lohuis, M., Vukasinovic, N., and Collier, R.J., Provisional application for United states letters patent for Single nucleotide polymorphisms and use of the same in selection of dairy cattle. Patent 37-21(53893) A.

19. Eenennaam, A.V., (2007). Marker assisted selection in beef cattle. Cooperative extension, University of California, Department of Animal Science.

20. Einspanier, R., Gabler, C., and Kloas, W. Expression of the beta 2 adrenergic receptor in cattle. Direct submission to Genbank, accession number Z86037

21. Franco, M.M., Antunes,, R.C., Silva, H.D., and Goulart, L.r., (2005). Association of PIT1, GH and GHRH polymorphisms with performance and carcass traits in Landrace pigs. Journal of Applied Genetics. 46(2): 195-200

22. Garcia-Sainz, J.A., Romero-Avila, M.T., Alcantara-Hernandez, R., Macias-Silva, M., Olivares-Reyes, A., Gonzales-Espinosa, C., (1992). Species heterogeinity of hepatic alpha 1-adrenergic receptors α_{1a}-, α_{1B}- and α_{1C} subtypes. Biochemical and Biophysical Research Communications. 186;760-767

23. Gether, U., (2000). Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. Endocrinology Review 21(1):90-113

24. Grant, A.L., Skjaerlund. D.M., Helferich, W.G., Bergen, W.G., and Merkel, R.A., (1993). Skeletal Muscle Growth and Expression of Skeletal Muscle actin mRNA and Insulin Like growth Factor I mRNA in pigs during feeding and withdraw of Ractopamine. Journal of Animal Science. 71: 3319-3326.

25. Gregory, K.E., Cundiff, L.V., and Koch, R.M., (1995). Genetic and phenotypic (Co)Variances for growth and carcass traits of purebred and composite populations of beef cattle. Journal of Animal Science. 73:1920-1926

26. Haegeman, A., Williams, J.L., Law, A., Van Zeveran, A., and Peelman., Journal of Applied Genetics L.J., (2003). Mapping and SNP analysis of bovine candidate genes for meat and carcass quality. Animal Genetics. 34: 349-353

27. Hale, D.S., Goodson, K., and Savell, J.W., (2004). Beef grading, http://meat.tamu.edu/beefgrading.html. Department of Animal Science, Texas

Agricultural Extension Service Texas A&M University, College Station, Texas.

28. Hammon, H.M., Bruckmaier, R.M., Honegger.,U.E., and Blum,J.W.(1994) Distribution and density of α and β-adrenergic receptor binding sites in the bovine mammary gland. Journal of Dairy Research. 61:47-57

29. Hancock, A.A., Delean, A.L., and Lefkowitz,R.J. (1979). Quantitative resolution of beta adrenergic receptor subtypes by selective ligand binding: application of a computerized model fitting technique. Molecular Pharmacology. 16:1-9

30. Hausdorff, W.P., Caron, M.G., and Leftkowtz, R.J., (1990). Turning off the signal: desensitization of β-adrenergic receptor function. FASEB J. 4:2881-2889.

31. Hermesmeyer, G.N., Berger, L.L., Nash, T.G., Brandt Jr, R.T., (2000). Effect of energy intake, implantation, and subcutaneous fat end point on feedlot steer performance and carcass composition. Journal of Animal Science. 78:1405-1420

32. Hieble, J.P., Bondinell, W.E., and Ruffolo Jr, R.R. (1995). Alpha- and beta-adrenergic receptors: from the gene to the clinic1. Molecular biology and adrenergic receptor subclassification. Journal of Medical Chemistry.38:3415-3444

33. Hoey, A.J., Reich, M.M., Davis, G., Shorthose,R., and Sillence, M.N., (1995). β_2-Adrenoceptor Densities Do Not Correlate with Growth, Carcass Quality, or Meat quality in Cattle. Journal of Animal Science. 73:3281-3286

34. Inderwies, T., Pfaffl, M.W., and Bruckmaier, R.M. (2003). Milking characteristics and their relation to adrenergic receptor mRNA expression and ligand binding in the mammry gland of dairy cows. Domestic Animal Endocrinology. 25:275-286

35. Inderwies, T., Pfaffl, M.W., Meyer, H.H.D., Blum, J.W., and Bruckmaier, R.M (2003). Detection and quantification of mRNA expression of α- and β-adrenergic receptor subtypes in the mammary gland of dairy cows. Domestic Animal Endocrinology. 24: 123-135

36. Inderwies,T., Riedl,J., Kiossis, E., and Bruckmaier, R.M (2003). Effects of α- and β-adrenergic receptor stimulation and oxytocin receptor blockade on milking characteristics in dairy cows before and after removal of the teat sphincter. Journal of Dairy Research. 70: 289-292

37. Koch, R.M., Cundiff, L.V., and Gregory, K.E (1982). Heritabilities and genetic, environmental and phenotypic correlations of carcass traits in a population of diverse biological types and their implications in selection programs. Journal of Animal Science. 55(6): 1319-1329

38. Kononoff, P.J., Deobald, H.M., Stewart, E.L., Laycock, A.D., and Marquess, F.L.S., (2005). The effect of a leptin single nucleotide polymorphism on

quality grade, yield grade, and carcass weight of beef cattle. Journal of Animal Science. 83:927-932

39. Kurose, H., and Lefkowtiz, R.J., (1994). Differential desensitization and phosphorylation of three cloned and transfected α_2-adrenergic receptor subtypes. Journal of Biological Chemistry. 269; 10093-10099.

40. Lafontan, M., and Berlan, M., (1993). Fat cell adrenergic receptors and the control of white and brownfat cell function. Journal of Lipid Research

41. Lomasney, J.W., Cotecchia, S., Lefkowitz, R.J and Caron M.G. (1991). Molecular biology of α-adrenergic receptors: implications for receptor classification and for structure-function relationships. Biochemical and Biophysical Acta. 1095:127-39.

42. Macherey – Nagel MN April 2005/Rev.04. Genomic DNA from Blood, User Manual, NucleoSpin® Blood

43. Marchello, J., Ahmadi, H., Duff, G and Schafer, D. Carcass merit of Wayguli cross cattle. Meat Science Laboratory, University of Arizona. http://ag.arizona.edu/ANS/research/research_04.html

44. Mersmann, J., (1998). Overview of the effects of β-adrenergic receptor agonists on animal growth including mechanisms of action. Journal of Animal Science 76;160-172

45. Meyer, H.H.D., (2001). Biochemistry and physiology of anabolic hormones used for improvement of meat production. Acta Pathologia, Microbiologia et Immunologica Scandinavica1090: 1-8

46. Miller, S.P., (2002). Beef cattle breeding programmes: Progress and prospects. 7th World Congress on genetics applied to livestock production. August 19-23, 2002, Montpellier, France.

47. National Research Council (1994) metabolic modifiers. Effects on the nutrient Requirements of Food Producing Animals. National Academy Press, Washington, DC.

48. Pietri-Rouxel, F., and Strosberg, (1995). Pharmacological characteristics and species related variations of β_3-adrenergic receptors. Fundamental Clinical Pharmacology. 9:211-218

49. Roets, E., Burvenich, R and Hamann, J (1995). Relationship between numbers of α_2 -and β_2-adrenergic receptors on blood cells of bulls and milkability of their daughters. Journal of Dairy Research. 62:567-575

50. Roets, E., Vandeputte-Van Messom, G., and Peeters, G., (1986). Relatiuonship between milkability and adrenoreceptor concentrations in teat tissue in primiparous cows. Journal of Dairy Science. 69(12):3121-3131

51. Roets, E., Vandeputte-Van Messom, G., Burvenich, C., and Peeters, G (1989). Relationship between numbers of α_2 and β_2 adrenoreceptors in teat tissue and blood cells and milkability of primiparous cows. Journal of Dairy Research. 72: 3304-3313

52. Sainz, R.D., and Hasting, E., (2000). Simulation of the development of adipose tissue in beef cattle. Pages 175-182 in modeling nutrient utilization in

farm animals. J.P McNamara, J. France, and D.E. Beever, ed. CABI Publishing, New York, NY.

53. Sainz, R.d., Kim, Y.S., Dunshea, F.R., and Campbell, R.G.(1993). Effects of ractopamine on pig muscle histology, calpains and beta adrenergic receptors. Australian Journal of Agricultural Research. 44:1441-1448.

54. SAS. 1998. User's guide: Statistics. SAS Inst.

55. Schenkel, F.S., Miller, S.P., Jiang, Z., Mandell, I.B., Ye, X., Li, H., and Wilton, J.W., (2006). Association of a single nucleotide polymorphism in the calpastatain gene with carcass and meat quality traits of beef cattle. Journal of Animal Science. 84: 291-299.

56. Schenkel, F.S., Miller, S.P., Ye, X., Moore, S.S., Nkrumah, J.D., Li, C., Yu, J., Mandell, I.B., Wilton, J.W., and Williams, J.L., (2005). Association of single nucleotide polymorphism in the leptene gene with carcass and meat quality traits of beef cattle. Journal of Animal Science. 83: 2009-2020

57. Schimpf, R.J., Schmutz, S.M., and Buchanan, F.C.,(2001). Genetic mapping of the ADRB2 gene on cattle chromosome 7. Animal Genetics. 32:390-393

58. Shi, A.G., ahmad, S., Kwan, C.Y., and Daniel, E.E (1989). Characterization of α-adrenoceptor subtypes by ^{3}H-prazosin and ^{3}H-rauwolscine binding to canine venous smooth muscle membranes. Canadian Journal of Physiology and Pharmacology. 67:1067-1073.

59. Sillence, M.N., Mathews, M.L., Spiers, W.G., Pegg, G.G and Lindsay, D.B., (1991). Effects of clenbuterol, ICI118551 and sotalol on the growth of cardiac

and skeletal muscle and β_2-adrenoceptor density in female rats. Archives of Pharmacology. 344:449-453

60. Smith, S.B., Garcia, D.K., and Anderson, D.B., (1989). Elevation of a Specific mRNA in longissimus muscle of Steers fed Ractopamine. Journal of Animal Science. 67:3495-3502.

61. Stiles, G.L., Caron, G.M and Lefkowitz, R.J., (1984). β -adrenergic receptors: Biochemical mechanisms of physiological regulation. Physiological Reviews. 64(2):661- 743

62. USDA, 1989. Official U.S. standards for grades of carcass beef. AMS, USDA, Washington, DC.

63. Utrera, A.R., and Van Vleck, L.D., (2004). Heritability estimates for carcass traits of cattle: a review. Genetics and Molecular Research. 3(3): 380-394

64. Valero, F.Y., Kobayashi, Y., Hoeffer Jr, C.A., and Collier, R.J (2002). Relationship of adrenergic receptor mRNA to milking rate in dairy cows. Plant, Animal and microbe Genomes X Conference, San Diego, California.

65. Vandewalle, B., Revillion, F., and Lefebvre, J., (1990). Functional β-adrenergic receptors in breast cancer cells. Journal of Cancer Research and Clinical Oncology. 116: 303-306

66. Venkataraman, V., Duda, T., and Sharma, R.K. (1997). The bovine α_{2D}-adrenergic receptor gene: structure, expression in retina, and pharmacological characterization of the encoded receptor. Molecular and cell Biochemistry.177: 113-123.

67. Vignal, A., Milan, D., SanCristobal, M., and Eggen, A., (2002). A review on SNP and other types of molecular markers and their use in animal genetics. Genetics Selection and Evolution. 34:275-305

68. Wang, S.Y., and Beermann, D.H., (1988). Reduced calcium dependent proteinase activity in cimaterol induced muscle hypertrophy in lambs. Journal of Animal Sciences. 66:2545-2550.

69. Wellnitz, O., Zurbriggen, A., Friis, R.R.., Blum, J.W., and Bruckmaier, R.M. (2001). α_{1c}-and β_2-adrenergic receptor mRNA distribution in the bovine mammary gland detected by competitive RT-PCR. Journal of Dairy Research. 68:699-704

70. Zhao, Q., Davis, M.E., and Hines, H.C., (2004). Associations of polymorphisms in the Pit-1 gene with growth and carcass traits in Angus beef cattle. Journal of Animal Science. 82: 2229-2233

71. ZoBell, D.R., Whittier, D., and Holmgren, L. (2005). Beef Quality and Yield grading. Practical Solutions for a Complex World. Extension Utah State University.

72. ZoBell, D.R., Whittier, D., and Holmgren, L., (2005). Beef quality and yield grading. Extension Utah University.