AN EVALUATION OF GENE INTERACTIONS AFFECTING CARCASS YIELD AND MARBLING IN BEEF CATTLE

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ABSTRACT

Genotype-specific management of beef cattle in feedlots has the potential to improve carcass uniformity. Gene variants affecting marbling include *LEPc.73C>T, ADH1Cc.-64T>C, TG5,* and *GALR2c.-199T>G* while those in *CRHc.22C>G, POMCc.288C>T, MC4Rc.856C>G* and *IGF2c.-292C>T* influence lean yield. The purpose of the current study was to assess combinations of marbling gene variants with those associated with lean yield and to investigate the effects of a gene variant in *serotonin receptor 1B* (*HTR1B*) on beef carcass traits. Gene variants were initially genotyped in 386 crossbred steers and evaluated for associations with carcass traits (hot carcass weight, average fat, grade fat and rib-eye area). The goal was to select a subset of variants to genotype in 2000 steers (1000 with hormone implants and 1000 without implants) with camera graded carcass data (Vision USDA yield grade, Vision grade marbling, rib-eye area and fat thickness). Seven gene variants were selected to proceed with (*TG* was discontinued) as they either had an association or were involved in gene interactions affecting a trait. In the implanted steers *GALR2* affected rib-eye area (P=0.002) where it exhibited an additive effect (TT=83.74 cm^2, TG= 84.32 cm^2 and GG=86.90 cm^2) however there was a dominant effect of the T allele for marbling (P=0.0001; TT/TG = 397.83 and GG=378.27) and fat (P=0.001; TT/TG=8.38 mm and GG=7.31). This same association with marbling (P<0.0001; TG/TT 463.52 mm and GG=430.90) and fat (P=0.006; TT/TG = 10.23 mm and GG=9.14 mm) was also observed in the non-implanted steers where again the T allele showed dominance. Gene-gene interactions affecting a trait were only observed in the non-implanted steers with the multivariate analysis: *LEPc.73C>T* and *IGF2c.-292C>T* with fat (P=0.05) and a trend with marbling (P=0.07); *MC4Rc.856C>G* and *POMCc.288C>T* with marbling (P=0.05); and *GALR2c.-199T>G* and *POMCc.288C>T* with rib-eye area (P=0.03). Associations between gene

variants with traits were made simpler due to the fact that some genotypes could be collapsed, as least square means (LSM) were not significantly different, indicating a dominant effect of one allele. The ability to pool genotypes not only simplified the interactions, it resulted in a larger number of animals with combined genotypes. The gene SNP networks generated using EPISNP support the mode of action between gene variants. For example, the gene interaction that was a 3 by 2 was also determined to be Additive-Dominance.

Significant associations were also identified between *HTRIB* c.205G>T SNP with carcass average fat (P=0.001), grade fat (P=0.007) and cutability (P=0.001) and a trend was observed with carcass REA (P=0.061). Although finding significance with several economically important carcass traits in crossbred beef breeds is novel, validating the effects of the *HTRIB* c.205G>T SNP in a larger cattle population would be beneficial.

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LIST OF ABBREVIATIONS

αMSH	Alpha Melanocyte Stimulating Hormone
β-END	Beta-endorphin
β-MSH	Beta-melanocyte stimulating hormone
γ-MSH	Gamma-melanocyte stimulating hormone
ACTH	Adrenocorticotrophin
ADH1C	Alcohol dehydrogenase 1C
AGRP	Agouti Related Protein
ANOVA	Analysis of Variance
CART	Cocaine-and-Amphetamine-Regulated Transcript
CAST	Collapsing and Summation Test
CBRH	Canadian Beef Reference Herd
C/EBPβ	CCAAT enhancer binding protein β
C/EBPδ	CCAAT enhancer binding protein δ
CFL	Cattleland Feedyards
CPM	Combinatorial Partitioning Method
CMC	Combined Multivariate and Collapsing Method
CRH	Corticotrophin Releasing Hormone
GALR2	Galanin Receptor 2
HCW	Hot Carcass Weight
HPA	Hypothalamic-Pituitary Adrenal Axis
HTR1A	Serotonin Receptor 1A
HTR1B	Serotonin Receptor 1B

HWD	Hardy-Weinberg Disequilibrium
IGF2	Insulin Growth-like Factor 2
IMF	Intramuscular Fat
LEP	Leptin
LD	Linkage Disequilibrium
LSM	Least Square Means
MAM	Marker Assisted Management
MC4R	Melanocortin-4-Receptor
MC3R	Melanocortin-3-Receptor
MDR	Multifactor Dimensionality Reduction
NPY	Neuropeptide Y
POMC	Pro-opiomelanocortin
PC1	Prohormone Convertase 1
PC2	Prohormone Convertase 2
QTL	Quantitative Trait Loci
REA	Rib-eye Area
REGWF	Ryan-Einot-Gabriel-Welsh F
SNP	Single Nucleotide Polymorphism
TBA	Trenbolone Acetate
TG	Thyroglobulin
VGMARB	Vision Grade Marbling
ZH	Zilpaterol Hydrochloride

1.0 GENERAL INTRODUCTION

North America's intensive livestock sector is continuously trying to improve the efficiency of their operations through a focus on animal performance and consumer satisfaction. Industry goals include providing the consumer with a consistent meat product of high quality, with characteristics that are considered desirable. A characteristic contributing to consumer satisfaction is marbling, otherwise known as intramuscular fat. It contributes to eating quality by influencing flavor and juiciness (Killinger *et al.* 2004; Wheeler *et al.* 1994).

Currently, beef carcasses are marketed in North America by either live or dressed weight, which focuses production on animal mass and detrimentally impacts meat quality through use of implants (Tronstad *et al*. 2005). Cattle sent to market after a visual assessment often leads to inconsistencies within animal pens. Producing carcass uniformity throughout the feedlot is a constant challenge in the level of finish when trying to find a balance between carcass quantity and quality (Nichols *et al.* 2014). While some animals would have the right amount of finish, others would be under or over finished (Woronuk *et al.* 2012).

As feedlots/producers focus on improving carcass quality through increased marbling, premiums are offered if the carcass grade falls within the predetermined requirements (Kononoff *et al.* 2005). The balance between lean yield and fat traits can be tenuous as one adversely impacts the other; therefore an agreed upon marketing strategy between feedlots/producers and slaughter facilities can provide a mutually beneficial relationship that also provides the consumer with the high quality beef product they want.

Improvement and uniformity of quantitative traits in livestock has been achieved by the selection of genes associated with a profitable phenotype (Snelling *et al*. 2012). Through the

study of gene interactions, it may be possible to determine the mechanisms that impact complex traits and make predictions to select animals based on their desirable carcass attributes (Snelling *et al*. 2012).

A number of candidate genes have previously been linked to carcass traits in beef cattle. Use of DNA tests with these candidate genes individually or through gene interactions could be a beneficial strategy to the beef industry by reducing variability in pens with respect to the level of finish of carcasses at slaughter. Another benefit comes from decreasing days on feed through the grouping of cattle by genotype, which enables the producer to target different markets (Van Eenennaam and Drake, 2012).

Phenotype variation occurs due to changes in the environment and how animals are managed. Different strategies that alter phenotypes include nutrition, housing and mating system design (Mulder *et al.* 2008). This topic is further complicated by the link between phenotype and genotype, with genes interacting with one another or other internal or external factors (Lewontin *et al.* 1992). Gene function can also be variable due to factors such as imprinting, interactions with other genes, gender and the environment. Altered gene function where no changes in the sequence of DNA occur is referred to as epigenetics (Anderson *et al.* 2012; Feinberg, 2007). The large amount of pen variability observed in economically important quantitative traits for livestock confirms that further assessment and analysis of gene interactions is necessary. However, currently single gene effects on traits are utilized. These assumptions ignore the contribution of multiple loci effects on phenotype variation (Templeton *et al.* 2000).

This thesis investigates the effect of gene interactions on carcass traits in beef cattle. It included the interaction of candidate genes that had previously been linked to lean yield or fat

traits, and the analysis of the new candidate gene Serotonin Receptor 1B (*HTR1B*) and its effect on carcass traits.

2. LITERATURE REVIEW

2.1 Marketing Cattle

Consumer demand and satisfaction with meat quality is commonly associated with desired characteristics such as the level of intramuscular fat, also known as marbling. This characteristic contributes to beef palatability, and influences flavor and juiciness (Killinger *et al.* 2004; Wheeler *et al.* 1994). Currently, marketing beef in North America is largely determined by either live or dressed weight using the average weight of the pen rather than individual measurements (Tronstad *et al*. 2005). This makes balancing carcass quantity, quality and uniformity a challenge in the beef industry (Nichols *et al.* 2014).

There has been a drive to improve animal performance in the feedlot and to provide consistent meat quality and characteristics that are considered desirable to the consumer. The consumer considers marbling to be perceived tenderness and is therefore a desirable meat characteristic (Killinger *et al.* 2004). As producers/feedlots work towards improving the marbling grade of their beef, premiums can be offered as a reward if the grade is within the requirements determined beforehand (Kononoff *et al.* 2005). The deciding factor in determining if cattle are ready to go for slaughter has previously been based on a visual assessment. Past use of this method would yield inconsistent levels of finish on the animals (Woronuk *et al.* 2012). Another issue producers are faced with is the balance between lean yield and fat traits, as one

will adversely impact the other, therefore producers must consider what marketing strategy will provide the greatest benefit for their operation.

Cattle that are marketed using the live weight method receive a price that is determined on a pen rather than individual basis (Tronstad *et al*. 2005; Parish *et al.* 2009). This strategy determines a price representing an average of carcass quantity, and is discussed between the feedlot/producer and packer before animals are sent to slaughter (Parish *et al.* 2009). Using this method of marketing cattle has a certain risk associated with it, as there is no drive to ensure that carcass quality is maintained which is a consumer preference (Tronstad *et al*. 2005).

A method used to satisfy consumer preference is formula value-based pricing (Tronstad *et al.* 2005). An example of this is grid pricing, and is a marketing strategy that establishes carcass value based on weight, yield and quality (Tronstad *et al*. 2005; Parish *et al.* 2009). Using standard carcass measures of Canada AA and Yield Grade 2 (Parish *et al.* 2009), the packer sets a fixed base price. Those carcasses that exceed the standard obtain premiums and discounts are given to those that fall below the set carcass measures (Parish *et al*. 2009). This strategy is similar to what is known as rail grade, where the producer is paid for value of the carcass, based on dressed weight and grade (Parish *et al*. 2009). The packer again sets a base price using carcass standards, and cattle that do not meet these requirements at slaughter receive a discount.

A strategy that can be utilized by feedlots to maximize their feed to gain ratio while still improving pen uniformity and meat quality is Marker Assisted Management (MAM). This approach incorporates managing cattle according to their genotype, where the feedlot/producer is able to sort, feed or breed their cattle to select for a desired trait (Van Eenennaam and Drake, 2012). Using this method allows the producer to achieve revenues by reducing days on feed and

grouping animals to target different markets, while improving uniformity per pen (Van Eenennaam and Drake, 2012). An example of a MAM that is currently being used commercially is a single nucleotide polymorphism (SNP) located within the leptin gene (Buchanan *et al.* 2002). The leptin SNP allows producers to select for TT animals that will enter the finishing stages earlier with more marbling in the meat, than in animals that are CC (Woronuk *et al.* 2012).

Creating a partnership between producers and feedlots for use of MAM may improve cost efficiency and carcass uniformity if the cost of DNA tests continues to fall and there is increased use of DNA testing in feedlots (Van Eenennaam and Drake, 2012). Selection of cattle based on their genetic potential could ultimately obtain the best value when sent to market.

2.2 Adipogenesis and Growth

Adipocytes are the primary cells that form white adipose tissue. Energy homeostasis is achieved by adipose tissue through triacylglycerol, which is mobilized and stored (Ali *et al.* 2013). Adipocytes have important functions in a number of physiological pathways where lipid and protein products are secreted. Evidence indicates that as a whole, adipocytes contribute to endocrine function which impacts appetite and tissue metabolism (Lefterova and Lazar, 2009). Other functions include the immune and inflammatory response, glucose uptake and the regulation of blood pressure and reproduction (Ali *et al.* 2013).

Maturation of adipocytes (adipogenesis) allows them to become fully functional (Lefterova and Lazar, 2009; Ali *et al.* 2013). This process is dependent on communication between cells and their environment, and is comprised of several phases. The first phase begins with a mesenchymal precursor that differentiates into a committed preadipocyte, which are

similar to fibroblasts (Lefterova and Lazar, 2009; Ali *et al.* 2013). The next phase halts growth of the preadipocytes by reducing proliferation, until hormones are released which initiates mitotic clonal expansion and the activation of CCAAT enhancer binding protein β (C/EBPβ**)** and CCAAT enhancer binding protein δ (C/EBPδ; Lefterova and Lazar, 2009; Ali *et al.* 2013). The transcription factors peroxisome proliferator-activated receptor γ (PPAR γ) and CCAAT enhancer binding protein α (C/EBP α) are generated, which activates expression of adipocyte genes (Lefterova and Lazar, 2009; Ali *et al.* 2013). The final phase of adipogenesis is actively expressing adipocyte genes and transcription factors PPAR γ and C/EBP α, which results in the lipid filled mature adipocyte (Lefterova and Lazar, 2009; Ali *et al.* 2013).

The adipose tissue found within the muscle between fiber bundles is defined as marbling in beef cattle (Albrecht *et al*. 2006). The amount of marbling is highly variable and is dependent on a number of factors, such as management and nutrition, maturity of the animal, gender, breed and genetics (Corbin *et al.* 2014; Panea *et al*. 2011). The regulation of carcass fat depots including intermuscular, intramuscular, subcutaneous and fat located around the internal organs represents an economic significance to producers, but is not well understood but may be important for improvement of carcass quality (Cianzio *et al.* 1985; May *et al.* 1995).

Growth rate is regulated by internal interactions that occur between a number of hormones and growth factors (Kononoff *et al.* 2005; Owens *et al.* 1993), which will differ, based on genotype, gender and breed differences. There are also external conditions that will affect growth rate, including how cattle are managed and fed, which can be limited by factors such as disease and poor weather (Kononoff *et al*. 2005; Owens *et al.* 1993).

2.3 Implants versus Non-implanted

The North American beef industry is continuously adapting methods to increase efficiency when confronted by consumer preference, changes in market access, cost of production and variable beef prices (Lopez-Campos *et al.* 2013). Use of growth implants and β − adrenergic agonists are a common practice found within our beef production system, with the best response observed with a mixture of hormones containing both estrogen and androgen compounds (Lopez-Campos *et al.* 2013; Reinhardt *et al.* 2007). However, recently there has been increased demand to produce beef raised without the use of hormones that promote growth (Wileman *et al.* 2009). Cattle producers are highly motivated to implement more efficient practices on their operations, and implanting their cattle allows them to improve animal growth despite the impact they have on fat deposition and meat quality (King *et al.* 2012).

A study conducted by Platter *et al.* (2003) utilized eleven treatment groups to determine how implants dispensed at different stages of production, effected carcass traits and consumer preference. The control group was not given implants at any stage of production for comparison to the other implanted treatment groups. Their results indicated that the non-implanted steers (the control group) marbled better than all other treatments groups except that there was no difference observed among treatment groups for the proportion of carcasses grading Choice or Prime (Platter *et al*. 2003). Implanted steers had a significantly higher shear force in comparison to the non-implanted steers (Platter *et al*. 2003). Consumers preferred non-implanted steaks and scored them higher for tenderness and flavor in comparison to all but one other treatment group (Platter *et al*. 2003). However, the implanted steers were heavier for final live weight, rib-eye area (REA) and hot carcass weight (Platter *et al*. 2003). Overall implications of this paper suggest that

certain implant protocols do adversely impact consumer preference and carcass quality, so producers should target certain markets based on their implant protocols (Platter *et al.* 2003).

Producers can increase profitability of their operations through use of commonly used implants such as estradiol benzoate and trenbolone acetate (TBA). This occurs due to the increase in carcass weights, muscle yield and feed efficiency (Lopez-Campos *et al.* 2013). However, quality grade suffers as marbling scores are decreased through intensive use of growth hormones (Platter *et al.* 2003). Beta-adrenergic agonists also increase protein synthesis by diverting nutrients away from fat deposition, which causes growth of muscle fibers (Lopez-Campos *et al.* 2013). The mechanism to increase protein accretion differs from β - agonists to growth hormones.

Growth hormones are able to alter gene function and stimulate changes in metabolic pathways. Trenbolone acetate plus estradiol effected transcription of several genes involved in a variety of metabolic pathways in livers of Nguni heifers (Becker *et al.* 2010). β -adrenergic agonists are also able to impact gene function. Zilpaterol Hydrochloride (ZH) is an example of a β -adrenergic agonist. Kononoff *et al.* (2013) studied its effect on leptin MAM and determined that it was inhibiting the effects of the T allele that would otherwise improve the marbling score, but no effect of ZH was observed in CC steers.

King *et al.* (2012) studied differing implant and management strategies to evaluate 47 SNPs that had known associations with carcass traits in cattle, and if favorable alleles were still effective under these practices. They also evaluated other economically significant carcass traits and if they were negatively impacted by different implant protocols. Their results indicated that although there were different implant protocols, the favorable alleles from the SNPs still

improved growth carcass traits. They concluded that MAM could be used in combination with implants to mitigate the adverse effects seen with more aggressive implanting strategies. Leptin for example, can be used in implanted animals where the effects of the SNP still improved the level of marbling (Woronuk *et al.* 2012). However, use of beta agonists such as Zilpaterol Hydrochloride reduced marbling in animals that had the TT genotype, but not those that were homozygous CC (Kononoff *et al.* 2013). Therefore beta agonists would not be used in TT animals. The previous examples demonstrate how gene function changes with growth hormone interactions, and challenges the industry to adapt.

Marketing decisions made by producers should take into consideration use of implants on their operations. Producers of non-implanted cattle may find it more economically feasible to target grid pricing for carcass quality, whereas feedlots that do implant their cattle could use a less aggressive protocol to avoid some of the adverse effects on tenderness and flavor (King *et al.* 2013; Platter *et al*. 2003).

2.4 Appetite and Stress Response Pathways

Appetite regulation is largely controlled by neuropeptides found in the arcuate nucleus of the hypothalamus, an area of the brain that is known as a key regulator for energy homeostasis (Ingvartsen and Boisclair, 2001). Neuropeptides that regulate feed intake have essential roles at different points during regulation of appetite, and must contend with other factors such as the individual's energy requirements and feeding behavior, which are dependent on the amount of adipose tissue, weight and the absorption of nutrients (Parker and Bloom, 2012). Neuropeptides signal a response between the hypothalamus and the gastrointestinal tract to create a negative

feedback loop that moderates appetite to meet an individual's metabolic demands (Parker and Bloom, 2012).

Found within the arcuate nucleus are two important pathways that contain anorexigenic and orexigenic neuropeptides, which regulate food intake and energy homeostasis. The first pathway (anorexigenic) decreases feed intake and includes pro-opiomelanocortin (POMC), cocaine-and amphetamine-regulated transcript (CART) and corticotropin-releasing hormone (CRH). The second pathway includes orexigenic neuropeptides that increase feed intake. These peptides are neuropeptide Y (NPY), agouti-related protein (AGRP), melanin-concentrating hormone and galanin (Ingvartsen and Boisclair, 2001).

Energy homeostasis is continually being challenged in order to respond to other demands, such as changes in availability of nutrients, body fat and weight (Parker and Bloom, 2012). For example, the stress response pathway is also regulated by neuropeptides that act to moderate homeostasis and suppress appetite (Parker and Bloom, 2012).

The hypothalamic-pituitary adrenal (HPA) axis is stimulated in response to a change in homeostasis by stressors, which leads to the synthesis and release of important hormones including CRH, adrenocorticotrophin (ACTH) and glucocorticoids. Specific organs that influence or release these hormones are the hypothalamus and hippocampus, the anterior pituitary and the adrenal gland (Parker and Bloom, 2012; Miller and O'Callaghan, 2002). An important function of CRH includes the activation of the stress response pathway. This response is initiated in the hypothalamus through receptors and is where CRH is synthesized and then released into the blood. It then binds to the CRH receptors found in the pituitary, thereby increasing the production and release of ACTH (Parker and Bloom, 2012). The increased

quantities of ACTH are circulated throughout the body that then act on the melanocortin receptor-2 in the adrenal gland. This subsequently produces and releases growth-inhibiting glucocorticoids, which act through a negative feedback loop that prevents further stimulation of the HPA axis and additional production and release of CRH and ACTH. This functions to maintain glucocorticoid homeostasis in the HPA axis (Miller and O'Callaghan, 2002).

Growth is inhibited by glucocorticoids through the increased production of leptin (LEP) which decreases the animal's appetite. LEP is transported to the hypothalamus after it is formed in white adipose tissue. This initiates a neural response of reduced feed intake with an increase in physical activity, and an increase is observed in the amount of energy used (Zhang *et al.* 1994; Houseknecht *et al*. 1998). When LEP is deficient, appetite increases and the obese phenotype is the outcome. This occurs when there is insufficient LEP to stimulate secretion of alpha melanocyte stimulating hormone (αMSH) in the hypothalamus (Forbes *et al*. 2001). Alpha MSH is a product of the pro-hormone POMC, which undergoes post-translational cleavage to form a number of peptides, including ACTH and alpha MSH (Parker and Bloom, 2012). Alpha MSH is essential in the process of reducing appetite as it binds to melanocortin-4-receptor (MC4R) neurons (Forbes *et al.* 2001). Once the production of LEP increases due to stimulation by glucocorticoids, the secretion of POMC increases and has the effect of increasing production of alpha MSH, which binds to MC4R and results in a decrease in feed intake due to a reduced appetite (Pritchard *et al.* 2002).

2.4.1 The *Serotonin Receptor 1B* Gene

Serotonin (5-Hydroxytryptamine) has long been associated with behavior and stress (Laporta and Hernandez, 2015). It also functions in energy metabolism with the release of neurotransmitters such as acetylcholine, dopamine and glutamate (Baldinger *et al.* 2015; Savli *et al.* 2012). Serotonin is synthesized throughout the body and binds to seven classes of receptors that are expressed in many tissues and organs. These signal through G-protein coupled receptor pathways and ligand gated ion channels (Laporta and Hernandez, 2015).

One of these receptors, 5-hydroxytryptamine receptor 1B *(*HTRIB), functions to regulate serotonin uptake and release in the dorsal raphe nucleus (Baldinger *et al.* 2015; Mekli *et al.* 2011). Similar to other receptors that bind serotonin, HTR1B impacts food intake, sleep and mood behaviors (Savli *et al*. 2012; Zhang *et al*. 2008). Past research has identified links between behavior and energy homeostasis that has led to an effect on cattle performance (Zhang *et al.* 2008). This link has been identified in other species as well, including mice (Rocha *et al.* 1998), humans (Lerer *et al.* 2006), dogs (Berga *et al*. 2004) and horses (Prause *et al.* 2007). In a study conducted on humans, HTR1B was linked to addiction behaviors in people with bulimia nervosa, body mass index and appetite (Levitan *et al.* 2001). Research conducted on *HTR1B* knockout mice resulted in increased food intake, weight gain (Bouwknecht *et al.* 2001) and aggressive behavior (Saudou *et al*. 1994), linking it to both the appetite response pathways and behavior.

2.5 Genes Affecting Growth

2.5.1. The *Leptin* Gene

Leptin (LEP) is the hormone product of the obese gene, and was originally identified in mice (Anton *et al*. 2010; Zhang *et al.* 1994). Research indicates that it is involved in maintaining body weight and is an essential component in appetite regulation (Anton *et al*. 2010). This occurs due to a lipostatic signal that is transported to the hypothalamus after it is formed and secreted from white adipose tissue (Anton *et al*. 2010). Leptin also has other roles such as in reproduction and immune function (Ehrhardt *et al*. 2001; Fantuzzi and Faggioni, 2000). The mechanism of its involvement in a feedback loop in the hypothalamus occurs in pathways that include insulin and glucocorticoids, where appetite is decreased and metabolism of energy is regulated (Delavaud *et al*. 2000). More energy is used when the leptin neural response suppresses feed intake while increasing thermogenesis and physical activity. In ruminants, there is a linear increase in levels of leptin as body fat and energy increases (Delavaud *et al.* 2000). An association was identified with leptin concentration and carcass adipose depots and carcass characteristics of beef cattle (Anton *et al*. 2010; Zhang *et al*. 1994; Houseknecht *et al.* 1998). Buchanan *et al*. (2002) discovered a SNP in exon 2 of bovine *leptin* (*LEP c. 73C>T*), that results in a non-conservative amino acid change from arginine to cysteine at residue four of the mature peptide. This additional cysteine likely affects the bioactivity of the protein through competition with the only existing disulfide bond (Buchanan *et al*. 2002). The T allele has been associated with increased fat deposition (Buchanan *et al.* 2002; Kononoff *et al*. 2005). This SNP is commonly used as a DNA test in feedlots, which allows the best value to be achieved from cattle as they are sent to market (Kononoff *et al.* 2005).

2.5.2. The *Alcohol Dehydrogenase 1C* Gene

Research indicates that intramuscular fat is impacted during the restriction of vitamin A levels (Ward *et al.* 2012; Gorocica-Buenfil *et al.* 2007). The enzyme alcohol dehydrogenase 1 (ADH1C) converts retinol to retinaldehyde which is then converted to retinoic acid, which modulates the expression of genes involved in adipogenesis (Molotkov *et al.* 2002; Ziouzenkova *et al*. 2007). Oka *et al.* (1998) identified that increased levels of marbling were observed with vitamin A restriction. A SNP identified in the promoter region of *alcohol dehydrogenase 1C* (*ADH1C* c. -64T>C) has been shown to impact carcass traits in beef cattle fed a restricted vitamin A diet, where animals with the TT genotype marbled 23% better than CC animals (Ward *et al.* 2012). The base pair change of a T to a C removes a binding site for CCAAT enhancer binding α (C/EBP), which is a transcription factor. This has potential for a reduction in the formation of retinoic acid and therefore reducing fat deposition in cattle with the CC genotype (Ward *et al.* 2012). The *ADH1C* c. -64T>C was thought to be a candidate for implementation as a DNA test in feedlots combined with reduced vitamin A, where carcass traits such as marbling could reach optimal levels and increase profitability of livestock operations (Ward *et al.* 2012).

2.5.3. The *Galanin Receptor 2* Gene

Galanin receptor 2 (GALR2) is a G-protein-coupled-receptor that is widely expressed in the body, which includes hypothalamus, spinal cord and a several other peripheral tissues such as the gastrointestinal tract (Lang *et al.* 2007). It is involved in initiating a number of stimulatory pathways such as the activation of signal transduction cascades (Lang *et al.* 2007). Another

pathway influenced by GALR2 is activation of phospholipase C, where Ca^{2+} is released through Ca^{2+} dependent chloride channels (Lang *et al.* 2007). Recent research indicates involvement in processes such as stimulation of the jejunum and the release of growth hormone, as well as alterations in feeding behavior (Kalra *et al.* 1999). The *GALR2*c.-199T>G SNP has been found to be associated with carcass traits in beef cattle, which includes an increase in average fat, grade, fat, marbling, cutability and rib-eye area (Madder *et al.* unpublished data). *GALR2* expression could be altered due to a change in the level of transcription through the introduction of a G allele instead of a T allele in the promoter region, which inserts a Sp1 transcription factor-binding site (Dynan and Tjian 1983). Although there is little known about the effects of *GALR2,* it's possible that this SNP contributes some function to the appetite pathway. More research is needed to establish the mechanism of *GALR2*c.-199T>G and its function in various pathways.

2.5.4. The *Corticotrophin Releasing Hormone* Gene

Corticotrophin Releasing Hormone (CRH) is a 41 amino acid neuropeptide whose main function includes the activation of the stress response pathway (Roche *et al*. 1988). This gene contains two exons and has been mapped to chromosome 14 in cattle (Barendse *et al.* 1997). The second exon encodes the prepro-CRH, whereas the first exon is not translated, but mRNA in the 5'untranslated region is encoded (Slominski *et al.* 2000). It has been identified as a factor inhibiting feeding behavior in rats (Morley and Levine, 1982) and mice (Rosenthal and Morley, 1989). Its role in stimulating the production and secretion of POMC, ACTH and MSH peptides has been well described (Miller and O'Callaghan, 2002; Slominski *et al*, 2000) and CRH has roles in other processes such as reproduction, the immune response, and appetite and energy

homeostasis (Slominski *et al.* 2000; Ingvartsen and Boisclair, 2001). This gene became a positional candidate for a quantitative trait loci (QTL) identified on chromosome 14 for post-natal growth (Buchanan *et al.* 2000). A SNP was identified (*CRH c*. 77C>G), that resulted in a non-conservative amino acid change from a histidine to aspartic acid (Buchanan *et al.* 2000). The *CRH c*. 22C>G SNP also resulted in a non-conservative proline to arginine amino acid change and was associated with an increase in carcass traits such as hot carcass weight (HCW) and REA in 256 Charolais crossbred steers (Buchanan *et al.* 2005), where presence of the G allele was correlated to higher yield.

2.5.5. The *Melanocortin-4 Receptor* Gene

The G-protein coupled receptor involved in the melanocortin pathway that is essential in moderating appetite is melanocortin 4 receptor (MC4R), located mainly in the hypothalamus (Du *et al.* 2013). Research has shown that MC4R maintains homeostasis and regulates body weight (Benoit *et al*. 2000) and has an important function in the appetite pathway, where once the peptide α-MSH has bound it causes a reduction in feed intake (Pritchard *et al.* 2002). Two variants have been identified in the bovine *MC4R* gene that has been shown to be associated with carcass traits (Buchanan *et al.* 2005; McLean and Schmutz, 2011). *MC4R c.856C>G* (changes the amino acid from leucine at position 286 to a valine) was genotyped in 256 steers with growth and carcass yield data where there was a trend with HCW (P=0.085; Buchanan *et al*. 2005). The variant in *MC4R g.989G>A* (changes the amino acid at position 330 from a serine to asparagine) was genotyped in 1367 steers where heterozygous animals had increased grade and back fat (McLean and Schmutz, 2011). Houston *et al*. (2004) identified an association with the

Asp298Asn SNP in pigs with backfat and feed conversion, and other SNPs link *MC4R* to body weight and other fat traits in several breeds of cattle (Zhang *et al*. 2009; Huang *et al*. 2010).

2.5.6. The *Pro-opiomelanocortin* Gene

POMC is a prohormone found in a number of different mammalian tissues such as the arcuate nucleus of the hypothalamus, the pituitary, skin, testis and immune system (Young *et al.* 1998). It is a complex gene that has many functions, including the stress response and appetite pathways (Slominski *et al*. 2000). Once the production of *LEP* increases due to stimulation by glucocorticoids, the synthesis and secretion of *POMC* increases which has the effect of increasing production of αMSH that once bound to *MC4R* reduces appetite (Pritchard *et al.* 2002; Buchanan *et al*. 2005). *POMC* encodes a number of peptides, including ACTH, beta-melanocyte stimulating hormone (β-MSH), gamma-melanocyte stimulating hormone (γ-MSH), and also β-endorphin (β-END; Pritchard *et al.* 2002). Alpha-melanocyte stimulating hormone is also encoded by POMC, and plays a significant role in the appetite pathway. A decrease in feed intake and appetite is due largely to the binding of α-MSH to MC4R or melanocortin 3-receptor (*MC3R*). The peptides γ-MSH and β-END also function to reduce appetite; however the effect of this is to a much smaller degree than α-MSH (Pritchard *et al.* 2002). As *POMC* passages through Golgi bodies within the regulated secretory pathway, posttranslational cleavages occur. Prohormone convertases (PC1 and PC2) cleave *POMC* and produce a number of bioactive peptides (Pritchard *et al.* 2002). A variant (*POMC* c.288C>T) that results in a silent mutation was genotyped in 256 Charolais crossbred steers where presence of the T allele increased ship weight and HCW (Buchanan *et al*. 2005). A second study (n=386) validated these results and

also found an increase in REA, with a decrease in average and grade fat with each subsequent T allele (Buchanan and Deobald, 2011).

2.5.7. The *Insulin Growth-like Factor 2* Gene

Insulin Growth-like Factor 2 (IGF2) is part of the insulin growth-like factor family, and is an important factor in fetal and muscle growth and myoblast proliferation and differentiation (Du *et al.* 2013). This 67 amino acid peptide has been mapped to chromosome 29 in cattle and is imprinted during fetal development where only the paternal allele is expressed (Goodall and Schmutz, 2007). Previous research has placed IGF2 in an important role in fetal and muscle growth (Reik and Walter, 2001), it increases muscle mass in pigs due to prenatal hyperplasia (Clark *et al.* 2014), and QTLs mapped to the chromosomal location of IGF2 in cattle have been identified for carcass and milk traits (Casas *et al*. 2003). Previous research by Goodall and Schmutz (2007) on the *IGF2 c*. -292C>T variant assessed whether muscle deposition was being effected in cattle and how expression of *IGF2* was regulated. The *IGF2 c*. -292C>T SNP does not result in an amino acid change as it occurs in the 5' region in a non-coding exon. Research by Goodall and Schmutz (2007) included three separate cattle populations. The first was the Canadian Beef Reference Herd (CBRH) of 143 animals where 29 were purebred and 114 were crossbred. The second population consisted of 146 yearling bulls of various breeds and the third cattle population consisted of 225 crossbred steers (Goodall and Schmutz, 2007). They identified a significantly larger REA in CC animals in the CBRH cattle that resulted in a 10% increase over the TT genotype. In the bull population, results indicated that more fat was deposited in CC animals, although there was no difference between genotypes with REA.

2.6. Gene Interactions and Methods of Analysis

Genomic selection has accelerated the improvement of quantitative traits in livestock and increased accuracy by targeting genes associated with a phenotype that producers can make profitable (Snelling *et al*. 2012). Analysis of quantitative data increases our knowledge about gene expression and genotype effect on phenotype to understand the underlying mechanisms that contribute to complex traits. Through the study of gene interactions, it may be possible to make predictions with more information to select livestock by carcass attributes and using a combination of causative polymorphisms (Snelling *et al*. 2012).

There are a number of factors that contribute to variation observed in phenotypes amongst individuals from the same population. Phenotypes are dependent on the environment, which can be altered by management strategy including nutrition and housing as well as the mating system used (i.e. crossbreeding; Mulder *et al.* 2008). However, the link found between phenotype and genotype points to an involved mechanism that is further complicated when genes interact with one another. Gene variation occurs in the presence of other genes or the effect of the internal and external environment of an individual (Lewontin *et al.* 1992). Interactions with genes and environmental variables can impact gene expression and is referred to as epigenetics (Anderson *et al.* 2012; Feinberg, 2007). Such effects may help us understand variability observed in the genetics of complex traits, as there are factors such as the environment and allele combinations, which will impact individual phenotypes. The large number of factors contributing to variability observed in quantitative traits confirms that it is necessary to develop methods to analyze gene interactions. Assumptions made by current models identify interactions according to their single gene effect on a trait, which may be overlooking the contribution of effects from multiple loci on phenotype variation (Templeton *et al.* 2000).

Identifying and characterizing gene interactions using standard parametric statistics can be challenging without an expansive database that enables combinations in genes with low allele frequency when testing for significance (Gilbert-Diamond and Moore, 2011). A small number of individuals in a genotype grouping make it difficult to accurately estimate the interaction effect. Examples using this scenario with logistic regression resulted in larger standard errors and incidence of type I errors (false positives) (Hosmer and Lemeshow, 2000). It is also possible to have a higher occurrence of type II errors (false negatives) with decreased power when genes with no significant main effect are not tested for an interaction, which can occur in the forward selection method (Gilbert-Diamond and Moore, 2011). Backward elimination does test all main effects and interactions, but needs a higher number of degrees of freedom. This is also an issue using stepwise analysis, but is generally a more flexible approach to the previous methods (Gilbert-Diamond and Moore, 2011). Although there are many ways to analyze data, it is clear that identifying significant gene interactions can be a challenge.

Linear regression is a parametric statistical method commonly used to analyze gene interactions with quantitative traits (Gilbert-Diamond and Moore, 2011). This method has several advantages, including ease of access and modeling, its defined results and developed assumptions (Gilbert-Diamond and Moore, 2011). When using linear regression for gene interaction analysis, a large sample size is necessary for parameter estimation when there are an increasing number of independent variables (Gilbert-Diamond and Moore, 2011).

There are certain collapsing approaches used in genetic analysis that can be used to test rare variants in genes that are combined, in the same pathway or in the same region of a chromosome (Dering *et al.* 2012). There are models that predict phenotype variation of multilocus genotypes using a method called the combinatorial partitioning method (CPM;

Gilbert-Diamond and Moore, 2011). This partitions phenotype variation and compares similarity between individuals while also looking for differences in partition means of the population to find causative SNPs that cause phenotype variation in economically important traits (Ma *et al*. 2008; Gilbert-Diamond and Moore, 2011). The CPM method interprets gene interactions based on allele combinations that may otherwise be missed by using a model only considering linear genetic effects (Ma *et a*l. 2008). A threshold level is determined prior to analysis where every genotype combination set is assessed for the proportion of explained variance for the traits of interest. This is known as the within- and between-partition variance strategy (Gilbert-Diamond and Moore, 2011; Nelson *et al.* 2001). A second threshold level determining minimum sample size per partition sets is also established to calculate enough degrees of freedom. This obtains more accurate estimates for the within-partition groupings (Gilbert-Diamond and Moore, 2011). Once these criteria are met, the partition sets undergo multi-fold cross validation for confirmation of accuracy. Interpretations about the effect of genotype on phenotype can then be made using simple parametric statistics from the genotype combination partitions (Gilbert-Diamond and Moore, 2011).

A computer program that uses some variation of the CPM method called EpiSNP utilizes genome wide association analysis to identify functional mutations (Ma *et al.* 2008). This will expand our knowledge of gene mechanisms and their function in various pathways to improve economically important traits (Gao *et al*. 2007). This program follows quantitative genetics where effects of gene interactions or single genes on complex traits are assessed through use of Fisher's (1918), Cockerham's (1954) and Kempthorne's (1954) methods. EpiSNP partitions interaction effects when linear single gene effects do not explain what is happening in the model (Ma *et al*. 2008). The mode of the effect between two interacting genes is identified through

analysis of allele x allele, allele x genotype, genotype x allele, and genotype x genotype interactions (Ma *et al*. 2008). This model also includes assumptions for linkage disequilibrium (LD) and Hardy-Weinberg disequilibrium (HWD) to test for interactions in populations where these assumptions may be present. These are measurements that interpret the interaction effect and determine allele combinations that have the most or least effect on the carcass trait. The goal of this program is to use genome wide analysis to create interaction networks that affect quantitative traits.

Although there are a considerable number of factors that influence complex phenotypes, knowledge of genetic mechanisms and gene interaction networks in combination with biological and molecular pathways can be advanced (Ma *et al*. 2008). Genomic selection improves traits and accuracy by identifying mutations that are associated with a trait that will allow a producer to make informed decisions about marketing livestock. However, more emphasis is needed for the analysis of gene interactions impacting complex traits. Novel SNPs and our current understanding of how genes function and interact allow us to understand how underlying mechanisms impact economically important traits.

2.7 Objectives and Hypothesis

The hypothesis was that marbling and lean meat yield will be simultaneously increased using a combination of two gene variants to improve overall carcass quality and consistency. The objectives were to evaluate the four gene variants (*LEP*, *ADH1C*, *TG* and *GALR2*) affecting marbling and the four gene variants (*CRH*, *POMC*, *MC4R* and *IGF2*) affecting carcass yield and to access gene variant combinations to determine if it is possible to simultaneously increase both lean meat yield and marbling.

The objectives for the chapter investigating the effects of serotonin receptor 1B on beef carcass traits were to screen the coding regions of *HTR1B* for gene variants, genotype the most promising variant in 386 crossbred steers and analyze for an association with carcass traits. Our hypothesis was that there would be an association between a variant in *HTRIB* with carcass traits.

3.0 AN INVESTIGATION OF GENE INTERACTIONS FOR BEEF CARCASS TRAITS

3.1 Introduction

Consumer satisfaction of Canadian beef is dependent on several factors, and includes intramuscular fat (IMF) or marbling, which contributes to overall eating quality (Corbin *et al*. 2014; Wheeler *et al.* 1994). Cattle producers have an opportunity to market their cattle at a premium if beef carcasses achieve high grades with optimal levels of fat cover (Pickworth *et al*. 2011). However, uniformity of beef carcasses going to market continues to be a challenge for the industry (Nichols *et al.* 2014). Carcass consistency and quality is impacted by a number of factors. Fat deposition for example, is dependent on nutrition and management, maturity, breed, gender and genetics (Corbin *et al.* 2014). These variables may also impact meat and fat color, carcass muscling, and fat coverage.

Analysis programs such as EpiSNP enable genome wide association analysis, which allows for the identification of mutations that will expand our knowledge of gene function to improve economically important traits (Gao *et al*. 2007). Epigenetic effects may help explain the variability observed in the genetics of complex traits, as there are factors such as the environment and allele combinations, which will impact individual phenotypes (Ma *et al.* 2008). Several quantitative genetics methods assessed the effects of gene interactions or single genes on complex traits (Fisher 1918; Cockerham 1954; Kempthorne 1954). Although there are a considerable number of factors that influence complex phenotypes, using knowledge of epigenetics and gene interaction networks in combination with molecular pathways may increase our understanding of genetics and its principle mechanisms (Ma *et al.* 2008).

Past research indicates the association of numerous candidate genes with yield and fat traits in beef cattle. *Leptin* (*LEP*; Buchanan *et al.* 2002), *alcohol dehydrogenase 1C* (*ADH1C*; when vitamin A is restricted, Ward *et al.* 2012), *thyroglobulin* (*TG*; Bennett *et al.* 2013), and *galanin receptor 2* (*GALR2*) are genes that influence marbling. Genes that affect lean meat yield are *corticotrophin-releasing hormone* (*CRH*; Buchanan *et al.* 2005), *pro-opiomelanocortin* (*POMC*; Buchanan *et al.* 2005; Deobald and Buchanan 2011), *melanocortin 4 receptor* (*MC4R*; Buchanan *et al.* 2005) and *insulin like growth factor 2* (*IGF2*; Goodall and Schmutz, 2007).

The beef industry could benefit from DNA tests using single genes, or through the use of a subset of genes that interact with one another. To determine gene effects on meat quality and quantity, we need to improve our understanding of molecular and biological mechanisms before we are able to improve carcass uniformity on commercial livestock operations (Gao *et al*. 2007).

3.2 MATERIALS AND METHODS

3.2.1 Animals

3.2.1.1. Pound-Maker Population

The Pound-Maker group of animals contained 386 crossbred steers that were purchased at an auction near Saskatoon, Saskatchewan in 2005. They were housed at the University of Saskatchewan (U of S) beef research facility during the backgrounding phase and at Pound-Maker Agventures (Lanigan, SK) for finishing before being slaughtered at XL Beef in Moose Jaw (Pugh *et al.* 2007). All steers received the same diets. Data used for analysis included carcass data namely hot carcass weight (HCW), average fat, grade fat, and ribeye area (REA).

Average fat is the average of three fat measurements collected along the 12th rib *longissimus dorsi* muscle and grade fat is the narrowest fat depth on the fourth quadrant.

3.2.1.2. Cattleland Feedyards Ltd. Population

A group of 2000 British crossbred steers were obtained at auction at an average weight of 274 kg. A group of 1000 animals were implanted with Component TE 100 and 1000 were non-implanted. Animals were shipped for slaughter to JBS Food Canada (Brookes, AB) when pen weight reached an average of 612 kg between June 3rd and July 31st, 2014. Data used for analysis included Vision Grade Marbling grade, REA, and fat thickness.

3.2.2. DNA Extraction

For the Pound-Maker animals a blood sample was collected from each animal (Pugh *et al.* 2011) and the DNA was extracted as described by Montgomery and Sise (1990). For the Cattleland Feedyards steers the ear tissue tag and DNA extraction method from Quantum Genetix (Saskatoon, SK) was utilized (Kononoff *et al.* 2013). Briefly tissue collected from the animals was cut into deep 96 well plates, where solution A consisting of 0.2 M NaOH was added. Plates were then incubated for 15 minutes at 58-62˚C, and then solution B, which consisted of 1.6% concentrated HCl and 0.1M Tris, was added to each well. The plates were then spun to ensure sufficient mixing of the solutions. DNA could then be used for subsequent PCR reactions.

3.2.3. Genotyping

Published gene variant tests in *LEP*, *ADH1C*, *TG*, *GALR2*, *CRH*, *POMC*, *MC4R* and *IGF2* (Table 3.1) were used, that had previously been shown to have an association with either lean meat yield or fat traits. These genes are not linked, and are inherited independently. Gene variants were first assessed in the Pound-Maker population of steers before proceeding to the 2000 head from Cattleland Feedyards Ltd.

Table 3.1. Published gene variant tests and their allele frequencies.

Gene	Gene variant	Allele Frequencies	Reference
LEP	*LEP* c.73C>T	T = 0.55	Buchanan *et al.*
		C = 0.45	2002
ADH1C	*ADH1C* c. -64T>C	T = 0.70	Ward *et al.* 2012
		C = 0.30	
TG	*TG*5	T = 0.70	Barendse *et al.* 1999
		C = 0.30	
GALR2	*GALR2* c. -199T>G	G = 0.56	Madder *et al*
		T = 0.44	Unpublished.
CRH	*CRH* c. 22C>G	G = 0.55	Pugh *et al.* 2011
		C = 0.45	
POMC	*POMC* c. 288C>T	C = 0.75	Thue and Buchanan
		T = 0.25	2003
MC4R	*MC4R* c. 856C>G	C = 0.66	Buchanan *et al.*
		G = 0.34	2005
IGF2	*IGF2* c. -292C>T	C = 0.72	Goodall and
		T = 0.28	Schmutz 2003

3.2.3.1. PCR-RFLP

The PCR primers and conditions along with restriction enzymes used for *LEP c.73C>T*, *ADH1C c.-64T>C*, *GALR2 c.-199T>G*, *POMC c. 288C>T*, and *MC4R c. 856C>G* are listed in Table 3.2. The total reaction volume was 25 µl and the PCR cocktail included 10X Taq NH_4SO_4 Buffer (Fermentas, Burlington, ON), 0.2 µM dNTPs (Burlington, ON), 2 M $MgCl_2$, 0.16 pmol of

the forward and reverse primers (Integrated DNA Technologies, Coralville, IA), 0.2 units Taq polymerase (Fermentas, Burlington, ON), and 100 ng of DNA template. The additives 1 M Betaine (Sigma-Aldrich, Oakville, ON) or 0.5 mg/ml BSA (New England Biolabs, Pickering, ON) were used (Table 3.2). A T100 Thermal Cycler (Biorad, Mississauga, ON) was used for PCR amplification. Initial denaturation occurred at 94˚C for 2 minutes. There were 35 cycles of 30 seconds at 94˚C, with 30 seconds at the annealing temperature (Table 3.2), and 45 seconds at 72˚C. A final extension at 72˚C occurred for 10 minutes, which finished with a hold at 4˚C. The products from the PCR amplification were digested with a restriction endonuclease (Table 3.2) followed the manufacturers' protocols before being separated on a 3% agarose gel.

Table 3.2. PCR-RFLP primers and genotyping protocols.

Gene Variant	Forward and Reverse Primers	Additive	Annealing Temperature (˚C)	Restriction Enzyme
LEP c.73C>T	ATGCGCTGTGGACCCCTGTATC TGGTGTCATCCTGGACCTTCC	BSA	56	*BspEI*
ADH1C c. -64T>C	CAGGGCTTAAAGATCCCAGA TAGCCAATGCTTGTCTCTCG	BSA	54	*BslI*
GALR2 c. -199T>G	AGGGCCAGGGAGCAGGAAC GGACACCGAGGACACGAG	Betaine	58	*BccI*
POMC c. 288C>T	GATGAGCAGCCGCTGACT GTCAGCTCCCTCTTGAATTCGAG	Betaine	52	*Bts$^{\alpha}$I*
MC4R c. 856C>G	TACCCTGACCATACTGATCG AGAGCAACAAATGATCTCTTTG	Betaine	52	*TaiI*

3.2.3.2. Real time PCR

A LightCycler 480 system (Roche Molecular Biochemicals) was used to perform the real time PCR of *CRH c.* 22C>G and *IGF2* c. -292C>T (Table 3.3) at Quantum Genetix (Saskatoon, SK). A duplex assay mix allowed for the simultaneous amplification of the gene variants. The

total master mix volume was 4 ul per reaction. This consisted of 5 U/ul of FroggaBio FastStart Taq DNA Polymerase (FroggaBio, Toronto), 10X PCR Buffer with $MgCl_2$, 10 mM dNTPs, 50 mM $MgCl_2$, 10 mM forward and reverse primer/4 mM TEX Cy5, 10 mM forward and reverse primer/4 mM FAM HEX, distilled water and 1ul of DNA.

The PCR conditions were as follows: denaturation at 95˚C for 10 minutes followed by cycles of 30 seconds at 95˚C, 30 seconds at 58˚C and 45 seconds at 72˚C. There was no final extension for these reactions.

Table 3.3. Real time PCR primers and probes.

CRH c. 22C>G	
Forward Primer	CGC CCG CTA AAA TGC G
Reverse Primer	CCA CCA GCA GGA CGC
Probes	Texas Red - ACT GCC GCT GCT CGT – BHQ2 Cy5 - ACT GCG GCT GCT CGT – BHQ2
IGF2 c. -292C>T	
Forward Primer	CCA CCT GGC AGT CGA G
Reverse Primer	CCC TGG GCG GTG GGT AAA GAG
Probes	FAM - ACC AGC GAC GTC CAG – BHQ1 HEX - CAC CAG TGA CGT CCA G – BHQ2

3.2.4. Statistical Analysis

3.2.4.1. Pound-Maker Population

To analyze the association between gene variants with carcass data, the MIXED procedure of SAS was used, where means were separated using the PDIFF statement.

$$Yij = \mu + GeneAi + GeneBj + GeneA \times GeneBij + eijk$$

Where Yij is the dependent variable for the i_{th} observation, μ represents the mean of the dependent variable, and eij represents the random error for each animal observed.

3.2.4.2. Cattleland Feedyards Population

The 2000 Cattleland Feedyards Ltd. (CFL) steers were randomly grouped by implant status, *ADH1C* genotype and two treatment levels of vitamin A (50% and 100% of the recommended NRC value), however, only an effect of implant status was observed. A separate analysis was conducted for each group; 1000 steers implanted and 1000 non-implanted.

3.2.4.2.1. Multivariate ANOVA

Gene variants that showed significance ($P<0.05$) for main effects (Appendix A) using the same equation conducted on the Pound-Maker steers were then analyzed at each carcass trait using a T-test to pool genotypes that were not significantly different. The pooling of genotypes was indicative of a dominant allele. Interactions could then be analyzed using the multivariate ANOVA GLM procedure of SPSS (IBM SPSS Statistics version 20). Gene interactions were established by pairing a gene significant for a fat trait with a gene significant for a lean yield trait. The T-test results were used to determine whether multivariate analysis would be conducted on a 2x2 (both genes had pooled genotypes), 3x2 (one gene had a pooled genotype) or 3x3 (neither gene had pooled genotypes) design. Means were separated using the REGWF (Ryan-Einot-Gabriel-Welsh F) test when neither gene had a pooled genotype. Significance was declared at $P<0.05$.

3.2.4.2.2. EPISNP

New programs such as EPISNP that are specific for gene interaction analysis have recently become available (Ma *et al*. 2008). To assess additive and dominance interaction effects for gene variants in the non-implanted and implanted steers, the serial computing program epiSNP1 was used. EPISNP1 statistics includes a general linear model with a two-step least squares analysis that tests individual and interaction SNP effects on carcass traits. The significance was tested using the F-test for single locus effects, and the model was as follows:

$$Yij = \mu + SNP + e$$

Where Yij is the dependent variable, μ represents the mean of the dependent variable, the SNP is the single locus genotype effect and e represents the random error. To determine the mode of effect for gene interactions, the single locus analysis results were partitioned into additive (A) and dominance (D) effects using the Cockerham and Kempthorne (1954) method. This allows us to interpret the gene combination effect between allelic means for each interaction. The model for testing interaction effects is as follows:

$$Yij = \mu + GeneA + GeneB + GeneA \times GeneB + e$$

Interaction effects were partitioned into AxA, AxD, DxA and DxD effects (Cockerham and Kempthorne, 1954), and significance was tested with a T test. The top 13 most significant interactions were selected to input into EPINET, a program found within EPISNP, which is used for the visualization of interaction networks between SNPs and carcass traits.

3.3. Results

3.3.1. Pound-Maker Population

3.3.1.1. Gene Variant Association Study

The Pound-Maker population was genotyped with the eight gene variants for the main purpose of selecting a subsample of genes to proceed with in the 2000 CFL steers. *LEP c.*73C>T significantly affected average fat (P=0.003; Figure 3.1) and grade fat (P=0.002). Animals homozygous for the T allele had significantly higher average fat and grade fat. However, it appears that the C allele was dominant as there was no significant difference between the CT and CC genotypes. Steers with both CT and CC genotypes had significantly lower fat in both traits compared to TT. The TT genotype for grade fat had a measurement of 9.30 mm, whereas CT and CC graded at 8.27 mm and 7.50 mm respectively.

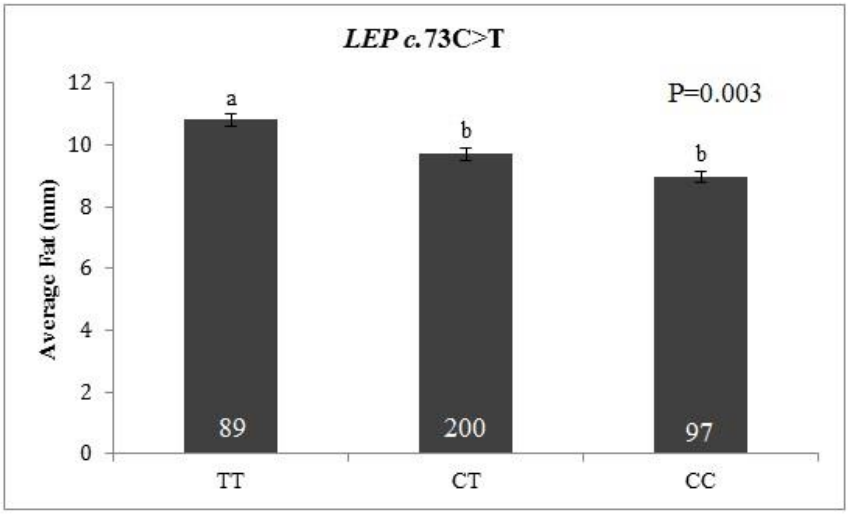


Figure 3.1. Least square mean (LSM) values for *LEP c.*73C>T genotypes with average fat in the Pound-Maker steers. The number of animals with each genotype is in each bar and standard error is shown.

GALR2 c. -199T>G was significantly associated with REA (P<0.001; Figure 3.2). Each G allele increased REA, where the GG genotype was approximately 11 cm^2 larger than the TT

genotype. Cattle in each genotype class were significantly different from one another, with the heterozygotes fell at an intermediate value between the homozygotes. This suggests an additive effect, where each addition of the G allele increased the size of REA.

POMC c. 288C>T was significantly associated with hot carcass weight (CC= 210, CT= 151 TT= 25; P=0.04). The significant difference was only between cattle with the CT and CC genotypes with weights of 381.41 kg and 373.64 kg respectively. Cattle with a TT genotype with a weight of 377.20 kg were not different from either the CT or CC steers, possibly due to the small number of animals present in that grouping (n=25) and the higher standard error of 1.47.

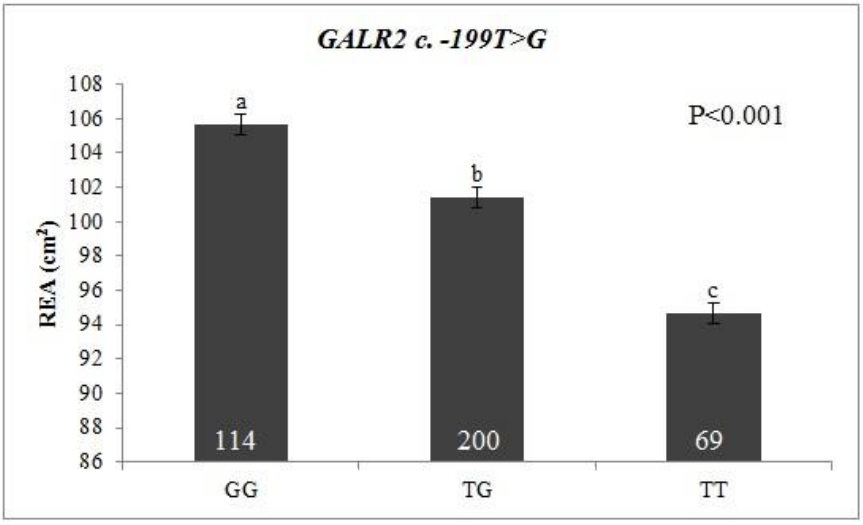


Figure 3.2. Least square mean (LSM) values for *GALR2 c*. -199T>G genotypes with REA in the Pound-Maker steers. The number of animals with each genotype is each bar and standard error is shown.

There were several significant gene interactions and others that showed trends with traits. The P-values for gene interactions are depicted in Appendix B. The *POMC c*. 288C>T and *GALR2 c*. -199T>G interaction was significant for average fat (P=0.01; Figure 3.3) and grade fat (P=0.05; graph not shown). The favorable genotype combinations were *POMC* CC and *GALR2*

TG and TT, *POMC* CT and *GALR2* TT, and with *POMC* TT and *GALR2* TT and TG. The *ADH1C c*. -64T>C and *POMC c*. 288C>T interaction was significant for average fat (P=0.05; Appendix C) and grade fat (P=0.03; graph not shown). The favorable genotype interaction between these two variants was TT for *POMC* and CT for *ADH1C*, however due to allele frequencies, there were only 11 animals in this group. The *GALR2 c*. -199T>G and *CRH c*. 22C>G interaction was only a trend with average fat (P=0.10; Appendix C). These graphs are obviously not easy to interpret and there are very low numbers of animals with the combined genotypes.

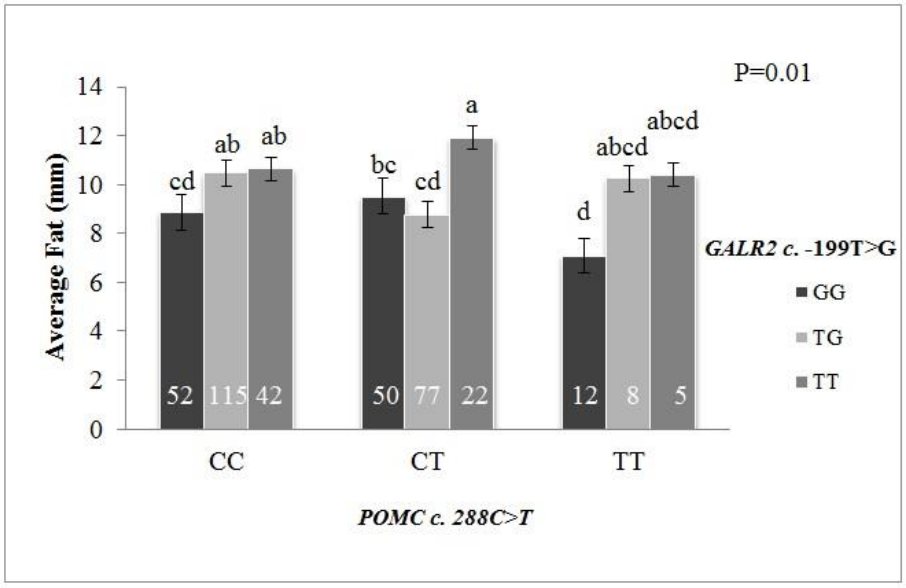


Figure 3.3. Least square mean (LSM) values for *GALR2 c*. -199T>G and *POMC c*. 288C>T with average fat in the Pound-Maker steers. The number of animals with each genotype is in each bar and standard error is shown.

Seven of the eight gene variants were selected to proceed with in the CFL steers, as they either had a trend, effect or were involved in gene interactions influencing a trait that demonstrated a trend which could possibly lead to significance in the CFL steers. Therefore the

only gene not selected to genotype was thyroglobulin, as it did not demonstrate any effect in the Pound-Maker steers.

3.3.2. Cattleland Feedyards Population

The allele frequencies for the gene variants genotyped in the CFL population are reported in Table 3.4. Several genes had moderate allele frequencies, such as *LEP* c.73C>T, *GALR2* c. -199T>G and *CRH* c. 22C>G. The remaining gene variants have frequencies that are skewed towards one allele.

Table 3.4. Calculated allele frequencies for gene variants genotyped in the 2000 steer CFL population

Gene	Gene variant	Allele Frequencies
Leptin	*LEP* c.73C>T	T = 0.55
		C = 0.45
ADH1C	*ADH1C* c. -64T>C	T = 0.74
		C = 0.26
GALR2	*GALR2* c. -199T>G	G = 0.51
		T = 0.49
CRH	*CRH* c. 22C>G	G = 0.51
		C = 0.49
POMC	*POMC* c. 288C>T	C = 0.75
		T = 0.25
MC4R	*MC4R* c. 856C>G	C = 0.70
		G = 0.30
IGF2	*IGF2* c. -292C>T	C = 0.80
		T = 0.20

3.3.2.1. Association Study

3.3.2.1.1. Non-implanted steers

Four gene variants significantly affected carcass traits in the non-implanted steers. The *GALR2 c*. -199T>G variant was significantly associated with vision grade marbling score and fat

thickness (Table 3.5). Cattle with the TT/TG genotypes, which demonstrate a dominance effect of the T allele, show a 7.6% increase in marbling score. Fat thickness follows the same pattern, where a 1.1 mm greater fat thickness was observed in the TT/TG steers compared to steers with the GG genotype.

Table 3.5. Least square mean (LSM) values for *GALR2 c*. -199T>G genotypes with Vision grade marbling and fat in the Cattleland Feedyards non-implanted steers.

	GALR2 c. -199T>G			
Variable	TT/TG (n= 643)	GG (n= 236)	SEM	P[1]
VGMARB	463.52[a]	430.90[b]	2.93	<0. 001
Fat (mm)	10.23[a]	9.14[b]	0.16	0.006

[1]Significance is P≤0.05. Only traits with a significant main or interaction effect are displayed. VGMARB=Vision Grade Marbling Score; Fat=Fat thickness; *GALR2=Galanin receptor 2*

A significant difference between steers with the TT/CT and CC genotypes for *IGF2 c*. -292C>T was observed for REA (P=0.01; Figure 3.4) where the T allele was dominant for this carcass trait. Steers that were CC at this locus had an increase of 1.94 cm^2 REA when compared to TT/CT steers. *POMC c*. 288C>T was significantly associated with fat thickness (P=0.001; Figure 3.5) between the TT/CT and CC genotypes. There was no significant difference between the TT/CT steers again indicating the T allele was dominant, which enabled them to be pooled together for analysis. The CC steers demonstrated a 1.24 mm increase in comparison to TT/CT steers.

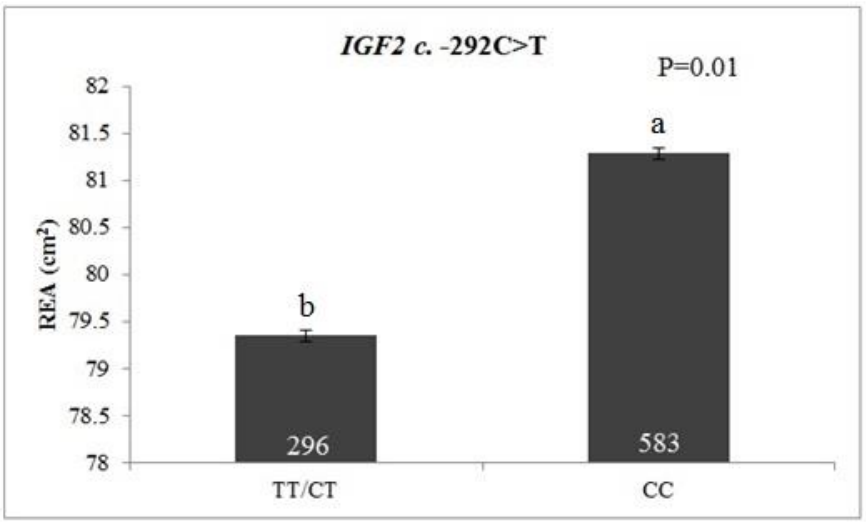


Figure 3.4. Least square mean (LSM) values for *IGF2 c*. -292C>T with rib eye area in the CFL non-implanted steers. The number of animals with each genotype is in each bar and standard error is shown.

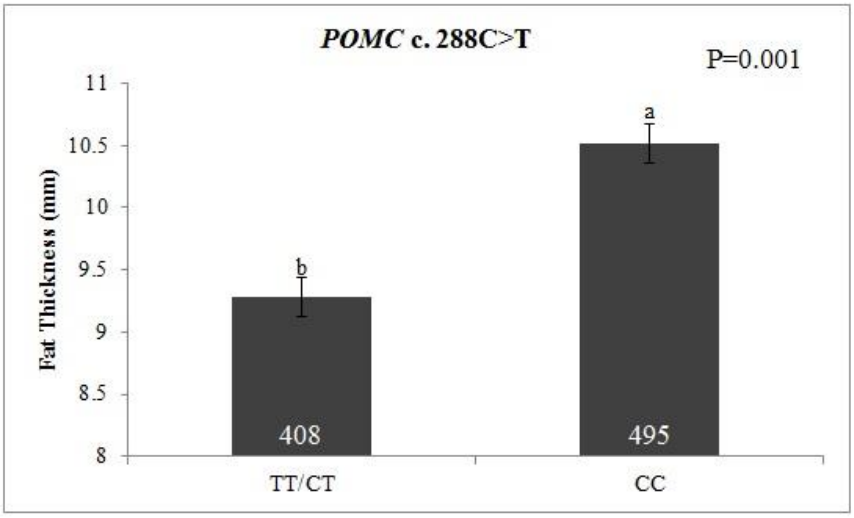


Figure 3.5. Least square mean (LSM) values for *POMC c*. 288C>T with fat thickness in the CFL non-implanted steers along with their main effect P-values. The number of animals with each genotype is in each bar and standard error is shown.

The *MC4R c*. 856C>G variant showed a significant association with fat thickness (P=0.034; Figure 3.6). There was no significant difference between steers with the GG/CG genotypes. The CC steers demonstrated a 0.84 mm increase when compared to animals with the GG/CG genotype.

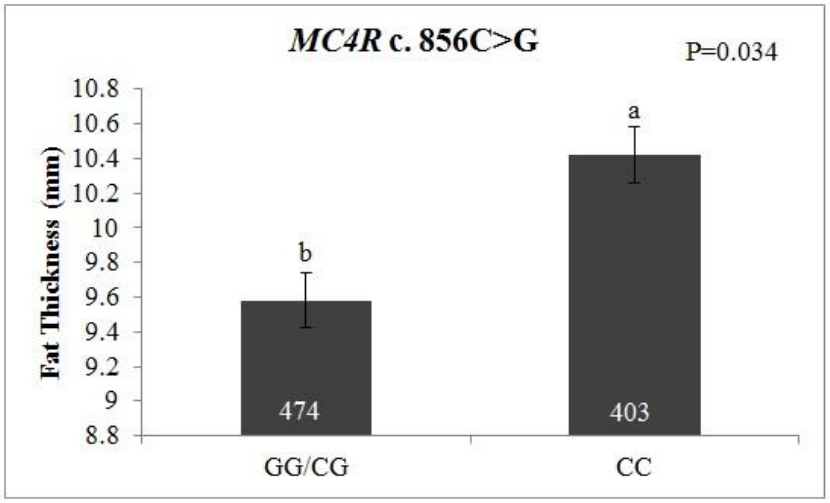


Figure 3.6. Least square mean (LSM) values for *MC4R c*. 856C>G with fat thickness in the CFL non-implanted steers along with their main effect P-values. The number of animals with each genotype is in each bar and standard error is shown.

The first of three interactions occurred between *LEP c*.73C>T and *IGF2 c*. -292C>T with fat thickness (P=0.05; Figure 3.7). The T allele for both variants was dominant; therefore the TT and CT genotypes for both variants could be pooled and analyzed as a 2x2 interaction. The favorable genotype was *IGF* TT/CT and TT/CT at *LEP* having significantly higher fat thickness with an increase of 1.83 mm from the smallest fat thickness measurement.

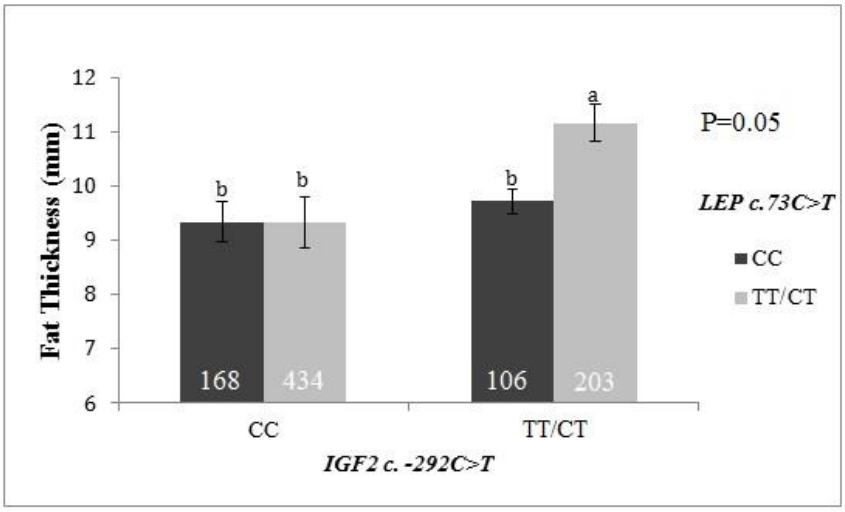


Figure 3.7. The *LEP c.*73C>T and *IGF2 c*. -292C>T interaction with fat thickness in the CFL non-implanted steers. The number of animals with each genotype is in each bar and standard error is shown.

The second interaction occurred between *POMC c. 288C>T* and *MC4R c. 856C>G* with vision grade marbling score (P=0.05; Figure 3.8). The T allele for *POMC c. 288C>T* and the G allele for *MC4R c. 856C>G* were identified as dominant and analyzed as a 2x2 interaction. The most favorable allele combination occurred at a score of 472.54 with CT/TT of *POMC* and steers with the CC genotype from *MC4R*, which had a score 6.8% higher than the lowest score of 442.53. There was no significant difference between cattle that had *POMC* CC and *MC4R* CC/CG/GG genotypes, or *POMC* TT/CT and *MC4R* GG/CG genotypes.

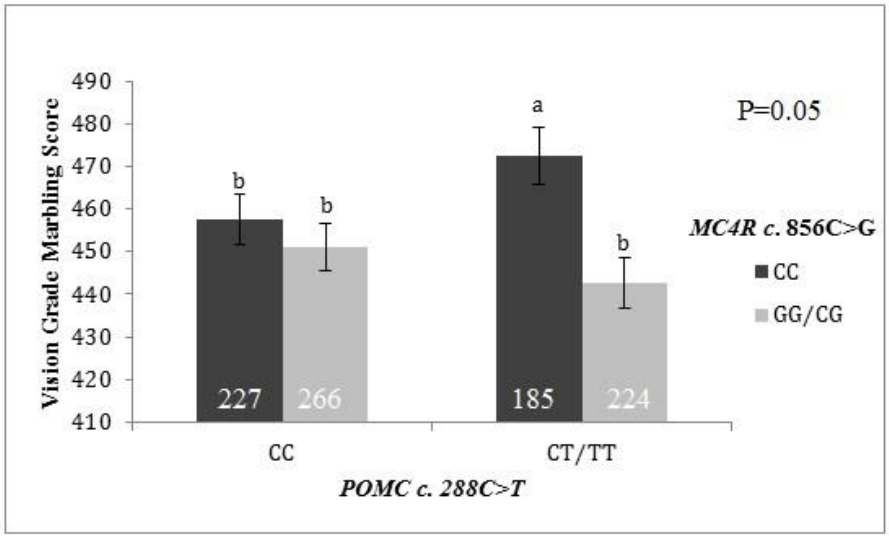


Figure 3.8. The *MC4R c. 856C>G* and *POMC c. 288C>T* interaction with vision grade marbling score in the CFL non-implanted steers. The number of animals with each genotype is in each bar and standard error is shown.

The last interaction occurred between *GALR2 c. -199T>G* and *POMC c. 288C>T* with REA (P=0.034; Figure 3.9). The T allele for *GALR2 c. -199T>G* was identified as dominant however, *POMC c. 288C>T* genotypes were not pooled together as no dominance pattern was present; therefore these variants were analyzed as a 3x2 interaction. The favorable allele combination appears to be TT/CT for *POMC c. 288C>T* and GG for *GALR2* c. -199T>G, with an increase of 9.68 cm^2 when compared to the lowest REA measurement of 78.58 cm^2. Cattle with the *GALR2* TT/TG in combination with CT or TT *POMC* genotypes shows a decreased REA when compared to steers with the GG genotype for *GALR2* with a T allele for *POMC*. When *GALR2* GG interacts with the *POMC* CC genotype, we see the same decreased REA as with the *GALR2* TT/TG genotype interactions with no significant difference present.

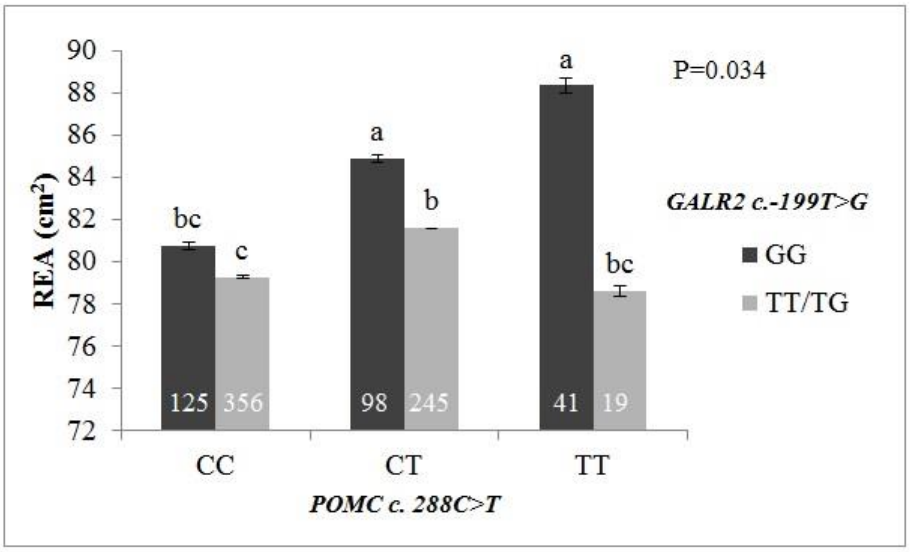


Figure 3.9. The *GALR2 c.-199T>G* and *POMC c. 288C>T* interaction in the CFL non-implanted steers. The number of animals with each genotype is in each bar and standard error is shown.

3.3.2.1.2. Implanted steers

In this group of steers we observed five gene variants associated with traits. *GALR2 c. -199T>G* was found to be significantly associated with vision grade marbling score (P=0.0001), fat thickness (P=0.001) and REA (P=0.002; Table 3.6). There was no significant difference between steers with the TT/TG genotypes for marbling score and fat thickness, therefore they were pooled together for analysis. Cattle with TT/TG genotypes for the marbling score is 5.2% higher and has about 1 mm thicker fat than cattle with the GG genotype. It is interesting to note that the fat traits are showing dominance of the T allele, but does not follow the same pattern for the REA trait, which was demonstrating an additive effect. For REA, the GG genotype in steers gives the largest measurement and in comparison to the TT steers results is a nearly 3.23 cm^2 difference.

Table 3.6. Least square mean (LSM) values for *GALR2 c*. -199T>G genotypes with Vision grade marbling, fat and REA in the Cattleland Feedyards implanted steers along with their main effect P-values

	GALR2 c. -199T>G			
Variable	TT/TG (n=670)	GG (n=242)	SEM	P[1]
VGMARB	397.83[a]	378.27[b]	1.96	0.0001
Fat (mm)	8.38[a]	7.31[b]	0.13	0.001
	TT (n=235)	TG (n=457)	GG (n=251)	
REA (cm^2)	83.74 [b]	84.32[ab]	86.90[a]	0.06	0.002

[1] Significance is P≤0.05. Only traits with a significant main or interaction effect are displayed. VGMARB=Vision Grade Marbling Score; Fat=Fat thickness; *GALR2=Galanin receptor 2*; REA=ribeye area

The vision grade marbling score was significantly associated with the *LEP* c.73C>T variant (P=0.04; Figure 10). Dominance of the T allele was observed for this trait, which was associated with a 2.8% increase in VGMARB compared to steers with the CC genotype.

IGF2 c. -292C>T was significant for fat thickness (P=0.01) and vision grade marbling score (P=0.007; Table 6). Fat thickness showed a difference of 0.66 mm between genotype groupings, with TT/CT animals having the larger measurement. The T allele for this trait showed dominance but when looking at the marbling score it became additive. The TT genotype has the largest marbling score when compared to CC, which has 7.1% less intramuscular fat than TT.

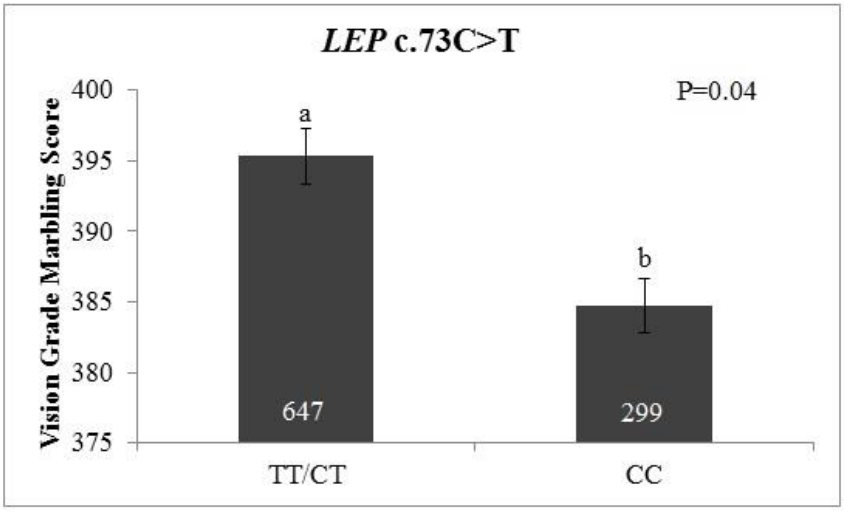


Figure 3.10. Least square mean (LSM) values for *LEP c.*73C>T in the CFL implanted steers along with their main effect P-values. The number of animals with each genotype is in each bar and standard error is shown.

Table 3.7. Least square mean (LSM) values for *IGF2 c.* -292C>T genotypes with Vision grade marbling and fat in the Cattleland Feedyards implanted steers along with their main effect P-values

IGF2 c. -292C>T					
Variable	TT/CT (n= 358)		CC (n= 576)	SEM	P[1]
Fat (mm)	8.48[a]		7.82[b]	0.13	0.01
Variable	TT (n=48)	CT (n=326)	CC (n=597)		
VGMARB	415.80[a]	394.11[ab]	388.15[b]	1.96	0.007

[1] Significance is P≤0.05. Only traits with a significant main or interaction effect are displayed. VGMARB=Vision Grade Marbling Score; *IGF2= Insulin Growth-like Factor 2*

The *POMC c.* 288C>T variant was found to be significantly associated with REA (P=0.02; Figure 3.11). The C allele demonstrated a dominance effect. The 56 cattle with the TT genotype exhibited a larger REA measurement by 3.74 cm^2. The *CRH c.* 22C>G variant was significantly associated with vision grade marbling score (P=0.02; Figure 3.12). Dominance was observed with the G allele, but it is the CC genotype that had the highest marbling score, which

was 3.1% greater than GG/CG. Again, no interactions were observed between gene variants in the implanted steer population.

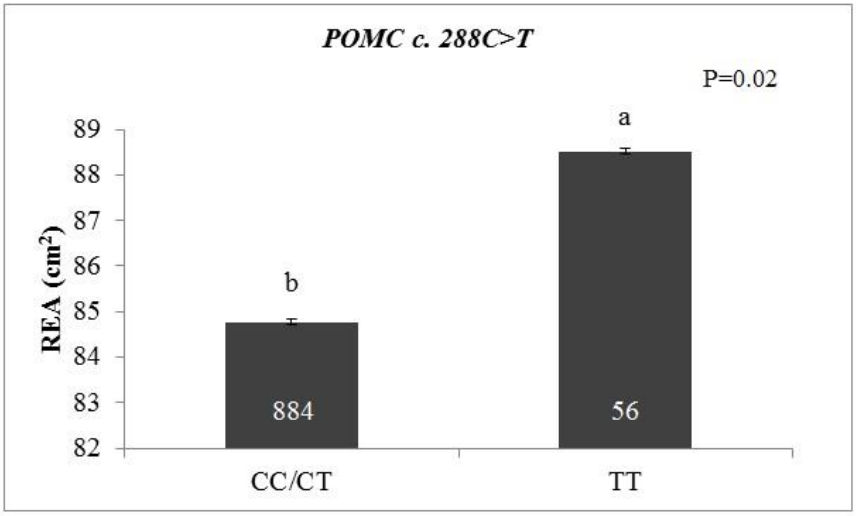


Figure 3.11. Least square mean (LSM) values for *POMC c*. 288C>T with REA in the CFL implanted steers along with their main effect P-values**.** The number of animals with each genotype is in each bar and standard error is shown.

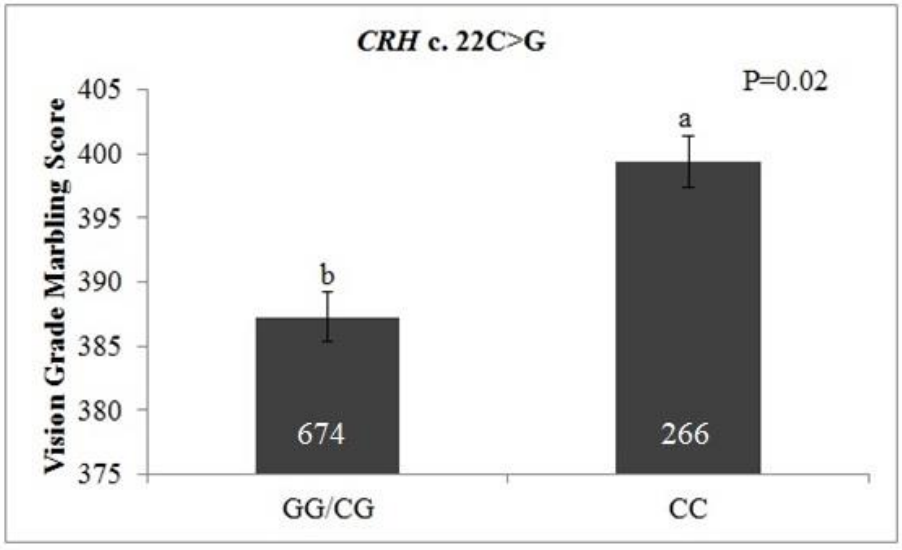


Figure 3.12. Least square mean (LSM) values for Vision Grade Marbling Score and *CRH c*. 22C>G in the CFL implanted steers along with their main effect P-values. The number of animals with each genotype is in each bar.

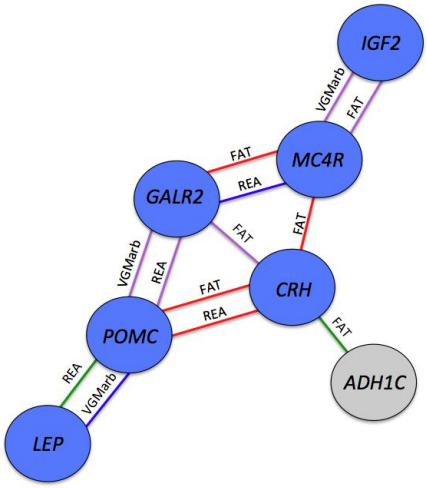
3.3.2.2. EPISNP

The following gene interaction networks were created using EPINET based on pairwise analysis result output from EpiSNP1. Each colored node indicates different levels of significance, where blue and grey are P<0.05 and P<0.10 respectively. Lines connecting nodes represent the following modes of interaction for carcass traits: green (DxD), blue (DxA), red (AxA) and purple (AxD). Directions of the interaction mode between each gene variant are listed in Appendix D. The term fat refers to fat thickness.

The non-implanted steer network (Figure 3.13) revolves around four significant central interconnected gene variants which include *GALR2* c. -199T>G, *POMC* c. 288C>T, *MC4R* c. 856C>G and *CRH* c. 22C>G. *GALR2* c. -199T>G and *MC4R* c. 856C>G interact with fat thickness (AxA) and REA (DxA). *GALR2* c. -199T>G and *POMC* c. 288C>T are connected by the traits vision grade marbling (AxD) and REA (AxD). *GALR2* c. -199T>G is connected to *CRH* c. 22C>G for the carcass trait fat thickness (AxD). *MC4R* c. 856C>G is the only variant to interact with *IGF2* c. -292C>T, and does so for the traits vision grade marbling and fat thickness (where both are AxD). *MC4R* c. 856C>G is also connected to *CRH* c. 22C>G, with an AxA mode of interaction for fat thickness.

CRH c. 22C>G is the only variant to interact with *ADH1C* c. -64T>C (DxD). Also, unlike all other variants in this network, *ADH1C* c. -64T>C is not significant, but was a trend. The variant *CRH* c. 22C>G is connected to *POMC* c. 288C>T through carcass traits REA (AxA) and fat thickness (AxA). The last interaction in this network is the only one to include *LEP* c.73C>T and occurs with *POMC* c. 288C>T for REA (DxD) and vision grade marbling score (DxA).

A



B

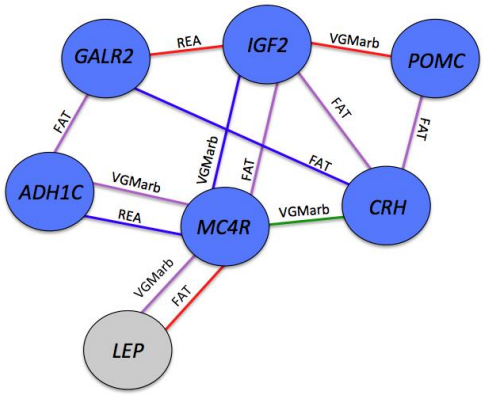


Figure 3.13. The top 13 most significant EPISNP results for interactions in *LEP*, *ADH1C*, *POMC*, *CRH*, *GALR2*, *MC4R* and *IGF2* with the Cattleland Feedyards steers. (A) SNP interaction network in the non-implanted steers (B) SNP interaction network in the implanted steers. Node colours indicate significance levels: blue (P<0.05) and grey (P<0.10). Lines connecting nodes represent modes of interaction for carcass traits: green (DxD), blue (DxA), red (AxA) and purple (AxD).

The implanted steer network contains interactions that occur less centrally around a subset of genes, but instead interacts more broadly with a larger number of variants. *GALR2* c. -199T>G interacts with three genes: *IGF2* c. -292C>T through a significant connection with REA (AxA), with *ADH1C* c. -64T>C for fat thickness (AxD) and with *CRH* c. 22C>G (DxA) for fat thickness. *IGF2* c. -292C>T interacts with *MC4R* c. 856C>G for vision grade marbling score (DxA) and fat thickness (AxD). *ADH1C* c. -64T>C and *MC4R* c. 856C>G have significant traits vision grade marbling score (AxD) and REA (DxA) connecting them. *LEP* c.73C>T is the only variant in this network that occurs in a single interaction. This occurs with *MC4R* c. 856C>G for vision grade marbling score (AxD) and fat thickness (AxA). *CRH* c. 22C>G interacts with *POMC* c. 288C>T (AxD) and *IGF2* c. -292C>T (AxD) for fat thickness, as well as with *MC4R* c.

856C>G for vision grade marbling score (DxD). *POMC* c. 288C>T and *IGF2* c. -292C>T interact with one another for vision grade marbling score with an AxA mode.

Interactions that occur between the same variants in both the non-implanted and implanted networks include *GALR2* c. -199T>G and *CRH* c. 22C>G for fat thickness, although the mode of the interaction differs, where it was AxD in the non-implanted steers, and DxA in the implanted steers. *MC4R* c. 856C>G and *IGF2* c. -292C>T interact in both networks for vision grade marbling and fat thickness, where the mode was AxD in the non-implanted animals for both traits, but vision grade marbling score becomes DxA in the implanted steers. Finally, *MC4R* c. 856C>G and *CRH* c. 22C>G interact for fat thickness, being AxA in the non-implanted network, but the carcass trait changes in the implanted steers to vision grade marbling score, with DxD being the mode of the interaction.

Interestingly, comparisons made between the two networks for the non-implanted and implanted steers demonstrates differences in interactions within the same subset of gene variants. The mode of the interaction can differ between the two populations for the same carcass trait. *GALR2* c. -199T>G and *CRH* c. 22C>G for example, are both significant for fat thickness, but in the non-implanted steers demonstrates an AxA mode, whereas this changes to DxA in the implanted steers. Also, the interaction between two of the same genes holds but the carcass traits affected changes. An example of this occurs between *MC4R* c. 856C>G and *CRH* c. 22C>G in the non-implanted steers with fat thickness but in the implanted steers the trait changes to vision grade marbling score. Also, both networks produced nodes for two different variants that were not significant and were only trends. In the non-implanted network, the trend was with *ADH1C* c. -64T>C, whereas in the implanted population it occurred with *LEP c*.73C>T.

MC4R c. 856C>G interacts with *IGF2* c. -292C>T in both networks for vision grade marbling and fat thickness. The mode of interaction for both of these traits is AxD in the non-implanted animals, but in the implanted steers, vision grade marbling score becomes DxA. The last interaction to occur in both networks is *MC4R* c. 856C>G and *CRH* c. 22C>G. The carcass traits connecting the variants in both networks differ. Fat thickness is significant and AxA in the non-implanted network, but in the implanted steers the carcass trait is vision grade marbling, which is a DxD mode of interaction.

3.4.3. Discussion

Genotyping of the Pound-Maker population determined which genes were selected to analyze in the CFL steers. Both populations were composed of British crossbred steers that were maintained on the same diet. A group of 1000 CFL and Pound-Maker animals were implanted in comparison to the 1000 non-implanted CFL steers. There were several main effects and interactions in the Pound-Maker population. The 3 by 3 interactions were hard to discern and the combined genotypes often resulted from a small number of animals due to skewed allele frequencies, for example between *GALR2 c*. -199T>G and *POMC c*. 288C>T with average fat (Figure 3.3). Others were associated with average fat but not grade fat. That was a concern, as typically both should be affected. There was also an interaction that was a trend (i.e. between *GALR2 c*. -199T>G and *CRH c*. 22C>G with average fat P=0.10; Appendix B) that we thought might become significant in a larger population.

There were a number of significant associations in the CFL steers that resulted in an increase in REA and fat thickness. These measurements can have a direct impact on yield grade

of the carcass. For example, an increase in fat thickness of the fourth quarter of the REA by 2.54 mm will impact the yield grade by 25% (Canadian Beef Grading Agency; United States Department of Agriculture, 2016). The yield grade is also affected, as REA size increases by 6.45 cm^2, a change in the yield grade occurs by approximately 30% (United States Department of Agriculture, 2016), directly affecting carcass value.

Cattle with a TT genotype at *LEP* c.73C>T showed a significantly increased level of average fat and grade fat compared to those with the dominant C allele. These results correspond with previous research where the T allele was associated with increased fat and the C allele with a leaner carcass (Buchanan *et al.* 2002). In the CFL implanted steers, the vision grade marbling score was associated with the *LEP* c.73C>T variant, but in this population the CT and TT genotypes were not significantly different which indicates dominance of the T allele. These results differ from the findings in the Pound-Maker population (C allele was dominant) and earlier studies (additive) performed on this variant (Buchanan *et al*. 2002; Woronuk *et al.* 2012). This research and previous literature supports that the T allele for *LEP c.*73C>T and the TT genotype is associated with a larger fat measurement the implanted steers.

GALR2 c. -199T>G was significantly associated with REA in the Pound-Maker steers and the implanted steers. An additive effect of each G allele was observed which increased the size of the REA. In both the non-implanted and implanted steers there was an association with vision grade marbling score and fat thickness where the T allele (exhibited dominance) increased marbling score and fat thickness. Although the 1.1 mm increase in fat thickness is statistically significant, it does not represent an economically significant value of 2.54 mm, according to the United States Standards for Grades of Carcass Beef. There is potential for expression of *GALR2* to be altered due to a change in the level of transcription through the introduction of a G allele at

the expense of a T allele in the promoter region, which inserts a Sp1 transcription factor-binding site (Madder *et al.* Unpublished; Dynan and Tjian, 1983). Although there is little known about the effects of *GALR2*, it's possible that this SNP contributes some function to the appetite pathway, allowing binding of galanin in the hypothalamus, stimulating feed intake (Parker and Bloom, 2012).

Cattle with a CC genotype in the non-implanted steers at *POMC* c. 288C>T showed a significantly increased level of fat thickness compared to cattle with a T allele. The implanted steers with a TT genotype at *POMC* c. 288C>T showed a significantly larger REA than cattle with a C allele. The TT/CT were pooled for fat thickness in the non-implanted (T allele dominance) while CC/CT were pooled for REA in the implanted cattle (C allele dominance). These results may indicate a role for *POMC* in metabolism and fat distribution (Forbes *et al.* 2001). The *POMC c*. 288C>T effect on REA in the CFL implanted steers resulted in TT steers having a larger REA by 3.74 cm^2. This may have a small impact on improving value of the carcass by increasing the yield grade of TT animals by approximately 15% (United States Department of Agriculture, 2016), however the frequency of this genotype is low and it may not be worthwhile to use MAM to select and manage these animals differently.

IGF2 c. -292C>T had a significant effect on REA in the non-implanted steers, where the CC genotype increased this trait. This finding was also reported in a crossbred steer population (n=135) and the Canadian Beef Reference Herd (CBRH; n=143) by Goodall and Schmutz (2007). The crossbred steers from this previous study demonstrate dominance of the C allele while in our study it was the T allele and in the CBRH there was an additive action. Increasing the size of the REA has potential to improve grading of the carcass by increasing lean yield, which would be economically beneficial to the producer (Goodall and Schmutz, 2007).

However, it is unlikely that 1.94 cm^2 would greatly increase the yield grade and value of the carcass. The CFL implanted steers demonstrated significance of *IGF2 c*. -292C>T with fat thickness, where the T allele was dominant, but the mode of inheritance becomes additive for the marbling score. Although this represents a significant difference in marbling that change from small to modest AAA, it does not result in a grade change to Prime beef. Goodall and Schmutz (2007) did not find any correlations to grade fat or marbling score in any of their studied cattle populations, although results from their bull population indicated percent fat to be associated with CC bulls, which does not align with our findings.

MC4R c. 856C>G only showed significance in the CFL non-implanted steers and was associated with fat thickness. It is possible that this difference in fat thickness between genotypes could impact the yield grade and therefore value of the carcass. However the CC steers only demonstrated a 0.84 mm increase when compared to animals with the GG/CG genotype, which may not be economically significant. Previous research identified this variant as a valine to leucine amino acid substitution, but only used growth and carcass yield data where a trend was found with HCW (Buchanan *et al*. 2005). However, another variant (*MC4R g*.989G>A) was associated with increased grade and back fat was found in a population of cattle heterozygous for the mutation (McLean and Schmutz, 2011), which supports the potential for *MC4R* to be used to improve fat traits in beef cattle.

The *CRH c*. 22C>G variant was only found to be significantly associated with vision grade marbling score in the CFL implanted steers, although the difference between genotypes is not enough to have any impact on changing quality grade to increase carcass value. A study conducted in 256 Charolais crossbred steers identified an association between *CRH c*. 22C>G, ribeye area and hot carcass weight (Buchanan *et al.* 2005), where presence of the G allele was

correlated to higher yield. Data for marbling or fat was not collected in this study, so direct comparisons cannot be made with the implanted steers. However, presence of the CC genotype in implanted steers and its correlation to increased fat and marbling does suggest that the GG genotype would increase yield.

3.4.1. Interaction Effects

Significant gene interactions were only observed in the CFL non-implanted steers. It is possible that interactions were not observed in the implanted steers because growth hormones induced changes in metabolic pathways, thereby altering gene regulation and function. A study conducted by Becker *et al.* (2010) analyzed the effect of trenbolone acetate plus estradiol on metabolic pathways and transcription in livers of Nguni heifers. Interestingly, their results indicated the down-regulation of *IGF2* and *IGF2* binding protein mRNA levels, which they suggest could increase movement of smooth muscle cells and disrupt lipid content. Research by Kononoff *et al.* (2013) identified an interaction between leptin and the β-adrenergic agonist Zilpaterol Hydrochloride (ZH). Where leptin would otherwise improve marbling scores for animals with the TT genotype, the ZH growth promotant acted as an inhibitor, and no difference in marbling score was observed between CT and TT genotypes. CC steers were not affected by ZH feeding.

Collapsing approaches used to predict phenotype variation of multilocus genotypes can be used where traditional analysis methods such as logistic regression provide less accurate results (Dering *et al.* 2012; Gilbert-Diamond and Moore, 2011). The dominant T allele for both *LEP c*.73C>T and *IGF2 c*. -292C>T enabled the TT and CT genotypes for both variants to be

pooled and analyzed together as a 2x2 interaction. A 1.83 mm increase in fat thickness was found to be associated with the *LEP c.*73C>T and *IGF2 c.* -292C>T interaction with TT/CT for leptin and TT/CT for *IGF2,* where 22.3% of steers in this population had the favorable genotype combination. This has potential to have an effect on yield grade, where a 2.54 mm increase represents a significant change of 25% (United States Department of Agriculture, 2016).

Analysis of *POMC c. 288C>T* and *MC4R c. 856C>G* took into account allele dominance by pooling genotypes. The interaction between these variants was significantly associated with vision grade marbling score. The TT/CT genotypes of *POMC* and the CC genotype from *MC4R* had a significantly higher marbling score of 472.54, which is classified as an AAA carcass grade. Within the non-implanted steers, 20.5% had the favorable genotype combination. By selecting the favorable allele combinations, producers could use the grid pricing marketing strategy that evaluates carcass quantity and quality (Parish *et al.* 2009), and consistently provide AAA cattle that exceed the set carcass standards to obtain premiums and minimize variation in pens sent for slaughter (Parish *et al.* 2009).

The dominant T allele of *GALR2 c. -199T>G* allowed for pooled genotypes while the *POMC c. 288C>T* genotypes were additive and hence these two variants were analyzed as a 3x2 interaction. The TT and CT of *POMC c. 288C>T* combined with GG at *GALR2* c. -199T>G steers (n=19 and 98 respectively) increased REA by approximately 9.68 cm^2 when compared to significantly different genotype combinations. This surpasses the 6.45 cm^2 standard that alters the yield grade by approximately 30% by a measure of 3.23 cm^2 (United States Department of Agriculture, 2016), and directly impacts carcass value.

POMC c. 288C>T had not previously been associated with REA (Buchanan *et al*. 2005). However, this trait was independently associated with *POMC* c. 288C>T and *GALR2 c*. -199T>G SNPs in the CFL implanted steers. Both alleles for *GALR2 c. -199T>G* are moderately heritable making this SNP a good candidate for use as a DNA test, due to its significance for both yield and fat traits. Low frequency of the T allele for *POMC c. 288C>T* does not make it an ideal candidate for DNA testing, however *GALR2 c. -199T>G* could be used as a single gene test. Producers could select GG cattle for *GALR2 c. -199T>G* to increase yield grade from a larger REA and reduce days on feed, or select TT/TG steers to improve fat traits. Specific markets could be targeted and uniformity per pen could be improved (Van Eenennaam and Drake, 2012).

3.4.2. EPISNP

The non-implanted and implanted steer data was used with the EPISNP program to produce gene interaction networks to identify the mode of interaction occurring between each gene variant. Interestingly, the EPISNP networks supported the mode of action observed between gene variants using the multivariate analysis of the pooled genotypes in the CFL steers. Pooling the genotypes resulted in a larger number of animals with the combined genotypes and simplified the interactions. The Additive-Dominance mode of *GALR2* c. -199T>G and *POMC* c. 288C>T for REA was observed in both multivariate analysis and EPISNP.

Changes in how gene variants are interacting with one another may be explained by the effect that occurs when alleles from one gene are combined with alleles from the second gene. This occurs in EPISNP as an extended version of the Cockerham and Kempthorne (1954) methods, where interaction effects are partitioned when linear single gene effects do not explain

what is happening in the model (Ma *et al.* 2008). This method determines the mode of the effect between two interacting genes through analysis of allele x allele, allele x genotype, genotype x allele, and genotype x genotype interactions (Ma *et al.* 2008). The mode of the interaction is also dependent on allele and genotype frequency, which will change depending on the combination of alleles occurring between genes in an interaction for a certain trait. These measurements interpret the interaction effect and determine allele combinations that have the most or least effect on the carcass trait (Ma *et al.* 2008).

Differences in how genes are interacting with one another may be due to the implant effect that is stimulating changes in metabolic pathways and changing gene function. This was observed with trenbolone acetate plus estradiol on metabolic pathways and transcription in livers of Nguni heifers (Becker *et al.* 2010) and the inhibition that occurs between leptin (TT genotype) and Zilpaterol Hydrochloride (a beta agonist), where no difference was observed between genotypes for the marbling scores between all genotypes when fed ZH for 21 days (Kononoff *et al.* 2013). However, more work with gene interactions and networks needs to be conducted to decisively conclude what is occurring between genes and complex carcass traits.

Changes in action can due to epigenetics (Anderson *et al.* 2012; Feinberg, 2007), which has potential to explain the variability seen between the Pound-Maker, non-implanted and implanted steers, as there are factors such as the environment and genotype combinations that effect individual phenotypes. It is possible that a combination of effects from multiple loci is contributing to variation occurring with carcass traits within and between cattle populations.

The complexity of phenotype variation is influenced by a number of factors that include gene interactions. The EPISNP networks identified associations between gene variants and

carcass traits, which furthers our understanding of quantitative traits and their underlying genetic mechanisms. However, the beef industry may find that there is a greater benefit through use of MAM with single gene tests versus interactions, which contain multiple genes and small animal numbers for favorable allele combinations. Further assessment of gene interaction use in a commercial setting should consider genes with moderate and equal allele frequencies to avoid small subsets of animals. This could be beneficial in identifying useful interactions that accurately estimate the effect of genes on carcass traits, therefore improving our ability to genetically select cattle.

4. AN INVESTIGATION OF SEROTONIN RECEPTOR 1B (*HTRIB*) ON BEEF CARCASS TRAITS

4.1 Introduction

Currently, marketing beef in North America is largely determined by either live or dressed weight, which may have a detrimental effect on meat quality (Tronstad *et al*. 2005). There has been a drive within the industry to improve animal performance in the feedlot and to provide consistent meat quality characteristics that are considered desirable to the consumer.

DNA tests are beneficial to the beef industry and enable the producer to improve efficiency of their operations. This is achieved by reduced variability in pens and decreasing days on feed by grouping their cattle to target different markets (Van Eenennaam and Drake, 2012). Mutations found in cattle DNA that impact meat quality can be amenable to genotype selection and precision management. A single nucleotide polymorphism (SNP) positively associated with a carcass trait allows for the sorting of animals to achieve different strategies of marketing cattle (Gao *et al*. 2007). The serotonin receptor 1B (*HTR1B*) may prove useful as a candidate gene for MAM by improving meat quality and quantity, as serotonin has been linked to behaviors such as sleep, mood and appetite (Zifa and Fillion, 1992).

The monoamine serotonin (5-Hydroxytryptamine) is involved in energy metabolism and the release of neurotransmitters such as acetylcholine, dopamine and glutamate (Baldinger *et al.* 2015; Savli *et al.* 2012). Receptors control levels of these neurotransmitters and serotonin through excitatory or inhibitory responses (Baldinger *et al.* 2015). Serotonin receptor 1A (HTR1A) for example, produces an inhibitory response on the serotonergic system through the prevention of postsynaptic cell firing and therefore the release of serotonin (Savli *et al.* 2012),

which is synthesized from the amino acid L-tryptophan, and forms 5-hydroxytryptophan after undergoing a rate-limiting step catalyzed by tryptophan hydroxylase (Laporta and Hernandez, 2015). Lastly, 5-hydroxytryptophan is converted to the final product, serotonin (Laporta and Hernandez, 2015). Past research has focused on neural functionality of serotonin, and its impact on behavior and stress. Serotonin production is widespread in the body and serotonin receptors are expressed in many tissues and organs, including the liver (Laporta and Hernandez, 2015). There are seven classes of serotonin receptors in mammals that regulate various functions, signal through G-protein coupled receptor pathways and ligand gated ion channels (Dass and Sudandiradoss, 2012; Laporta and Hernandez, 2015).

HTRIB is found within the G-protein coupled receptor family and regulates uptake and release of serotonin from the dorsal raphe nucleus (Baldinger *et al.* 2015; Mekli *et al.* 2011). Like many other serotonin receptors, *HTR1B* has been linked to appetite (Levitan *et al.* 2001), sleep and mood behaviors in humans (Ickowicz *et al.* 2007), but is also involved in regulating the activity of *HTR1A* (Savli *et al.* 2012). *HTR1B* is able to impact cattle performance, and associations with behavior and energy homeostasis have been established (Zhang *et al.* 2008). In mice (Rocha *et al.* 1998) and humans (Lerer *et al.* 2006) it has been linked to addiction behaviors and in people with bulimia nervosa, body mass index and appetite (Levitan *et al.* 2001). Through use of *in situ* hybridization, Bruinvels *et al.* (1993) was able to detect *HTRIB* mRNA levels in the caudate-putamen and cortex, as well as in the hippocampus, cerebellum and cerebral arteries of rodents. The bovine *HTR1B* gene is located on cattle chromosome 9 and encodes a 389-amino acid polypeptide (Zhang *et al.* 2008).

In humans, there have been many *HTR1B* polymorphisms identified in the coding sequence and untranslated regions with linkage to a schizophrenia susceptible gene (Sanders *et*

al. 2002) and a number of psychiatric conditions, such as depression, anxiety and eating disorders (Savli *et al.* 2012). The *HTR1B* G861C polymorphism for example, was found to be associated with minimum lifetime body mass index in women diagnosed with bulimia nervosa (Levitan *et al.* 2001). Polymorphisms identified in the serotonergic system have also been found to occur in other species such as the dog (Berga *et al.* 2004) and in horses where a possible link with gastrointestinal disorders was identified (Prause *et al.* 2007). In studies where the *HTR1B* gene was knocked-out, mice demonstrated behaviors such as increased food intake and weight gain (Bouwknecht *et al.* 2001). These mice also showed an increase in aggressive behavior (Saudou *et al.* 1994).

Previous research of *HTR1B* gene function demonstrates an association between animal behavior and energy homeostasis (Zhang *et al.* 2008), and has potential to impact livestock carcass traits. The aims for this chapter were to screen coding regions of the candidate gene *HTR1B* for gene variants, devise a DNA test to genotype 386 cross breed steers and analyze for an association with carcass traits.

4.2 Materials and Methods

4.2.1. Animals

This group of animals consisted of 386 crossbred steers that were bought at auction near Saskatoon, Saskatchewan in 2005. They were housed at the University of Saskatchewan (U of S) beef research facility and at Pound-Maker Agventures during backgrounding and finishing respectively. DNA had previously been extracted from a blood sample collected from these animals and followed the protocols of Montgomery and Sise (1990) and Pugh *et al.* (2011). All steers received the same diet and were slaughtered at XL Beef in Moose Jaw (Pugh *et al*. 2007).

Data used for analysis included hot carcass weight (HCW), average fat, grade fat, REA, and cutability. The average fat carcass trait is comprised of taking three fat measurements of the 12th rib *longissimus dorsi* muscle. Grade fat is measured by looking at fat depth of the fourth quadrant for the narrowest section, and cutability is an estimate of carcass yield.

4.2.2. Gene variant identification

Genomic sequence of the *HTR1B* gene (1,167 base pairs) was amplified and sequenced in 16 animals. Primer3 software (Untergrasser *et al.* 2012) was used to design primers with the *Bos taurus* genomic reference sequence found in GenBank (shotgun sequence; NC_007307.6). Sequencing was conducted at the Plant Biotechnology Institute (Saskatoon, SK) and was analyzed for SNPs using Sequencher 4.9 (Genecodes, Ann Arbor, MI) software.

4.2.2.1. Genotyping HTR1B c.205G>T

HTRIB c.205G>T was genotyped using sequence. The total reaction volume was a 25 µl PCR cocktail that included 10X Taq NH_4SO_4 Buffer (Fermentas, Burlington, ON), 0.2 µM dNTPs (Burlington, ON), 2 nM $MgCl_2$, 0.16 pmol of the forward (5'CCA GAC TGG GCT TTC TCA AG 3') and reverse (5'CTA ACT GCG TGG TGA AAC ACC 3') primers (Integrated DNA Technologies, Coralville, IA), 0.2 units Taq polymerase (Fermentas, Burlington, ON), and 100 ng of DNA. The additive 1M Betaine (Sigma-Aldrich, Oakville, ON) was used in this reaction. A T100 Thermal Cycler (Biorad, Mississauga, ON) was used for PCR amplification. Initial denaturation occurred at 94˚C for 2 minutes. There were 35 cycles of 30 seconds at 94˚C, with 30 seconds at the annealing temperature 56˚C, and 45 seconds at 72˚C. A final extension at

72˚C occurred for 10 minutes, which finished with a hold at 4˚C and was stored at -20˚C. The amplicons were then separated on a 1% agarose gel for quantification using a low mass DNA ladder (Invitrogen), and 10 µl of the remaining product was combined with 1µl exonuclease 1 and 2 µl saspAP, and DNA was sent for sequencing at Plant Biotechnology Institute (Saskatoon, SK). The sequence was then analyzed using Sequencher 4.9 (Genecodes, Ann Arbor, MI) software to determine animal genotypes.

4.2.3. Statistical Analysis

To analyze the association between the *HTRIB* c.205G>T variant and carcass data, a one-way analysis of variance (ANOVA) was conducted using the General Linear Model (GLM) procedure of SPSS to determine the least-square (estimated marginal) means. Means were separated using the Ryan-Einot-Gabriel-Welsh F (REGWF) test.

The following model was used to test the effect of the *HTR1B* genotypes on carcass traits:

$$Yij = \mu + HTR1Bi + eij$$

Where Yij is the dependent variable for the i_{th} observation, µ represents the mean of the dependent variable, and eij represents the random error for each animal observed. *HTR1B*i is the effect that *HTR1B* genotypes have on the dependent variable. Significance was declared at $P \leq 0.05$.

4.3. RESULTS

4.3.1. *HTR1B c.205G>T*

Two SNPs were identified in HTR1B's single exon that is 1,167 base pairs long. The first SNP located 205 base pairs from *HTR1B's* start codon. This resulted in a guanine to thymine base pair change. The second SNP, located 546 base pairs from the start codon altered the sequence from a cysteine to a guanine. The *HTRIB c.205G>T* was selected for genotyping in the 386 steers. This decision was based on the allele frequency observed in the 16 animals that were sequenced and because it was a missense mutation. The change in sequence results in a non-conserved amino acid change from alanine (nonpolar) to a serine (polar) at amino acid 69. The second SNP was a silent mutation as it did not result in an amino acid change; therefore it was not selected for genotyping. The allele frequencies for *HTRIB c.205G>T* were 0.67 and 0.33 for the G and T allele respectively. Genotype frequencies were 0.44 for GG (n=130), 0.45 for GT (n=177) and 0.11 for TT (n=21).

4.3.1.1. Association Study

HTRIB c.205G>T was significantly associated with average fat (P=0.001), grade fat (P=0.007), cutability (P=0.001) and there was a trend with REA (P=0.061; Table 4.1). The T allele is acting dominantly in all of these traits as there is no difference between GT or TT genotypes. Cutability was significantly increased in steers with one or two T alleles compared to GG. Carcass REA was also numerically larger when a T allele was present. The recessive genotype (GG) significantly increased average and grade fat measurement. The TT and GT

genotypes for the fat traits were statistically different from GG, but are not statistically different from one another. Presence of a T allele decreases average and grade fat significantly when compared to the presence of both G alleles. For optimal measurements in fat traits, GG is the genotype of choice, whereas having one or both T alleles seems to be favorable to improve yield.

Table 4.1. Least square mean (LSM) values for *HTR1Bc.205G>T* in the steers along with their main effect P-values

	HTR1Bc.205G>T				
Variable	GG n=130	GT n=177	TT n=21	SEM	P-Value[1]
Average Fat (mm)	10.53[a]	9.35[b]	8.14[b]	0.198	0.001
Grade Fat (mm)	8.94[a]	7.88[b]	6.95[b]	0.197	0.007
Cutability (%)	60.43[b]	61.61[a]	62.10[a]	0.173	0.001
REA (cm^2)	99.54	102.48	103.86	0.652	0.061

[1] Significance is $P \leq 0.05$.

4.4. Discussion

The SNP is a G to T transversion 205 base pairs from the start codon and elicits a non-conserved amino acid change from alanine (nonpolar) to a serine (polar). Zhang *et al*. (2008) previously identified this SNP in Chinese Holsteins. Although this is not a novel variant, identifying it in beef cattle and finding associations with carcass traits is valuable. The alanine to serine amino acid change likely alters the folding of the HTR1B protein affecting its bioactivity. The 69th amino acid is located early in the transmembrane region, and alanine is conserved in other mammalian species, such as the dog, human, horse and pig (Zhang *et al*. 2008).

Allele frequencies for *HTRIB* c.205G>T in the steer population was 0.67 and 0.33 for the G and T allele respectively. Zhang *et al.* (2008) reported allele frequencies of 0.75 for the G allele and 0.25 for the T allele in 61 Chinese Holsteins. The differences in allele frequencies between both populations are small and likely reflect breed differences.

Traits important to carcass quality that were significantly associated with the *HTRIB* c.205G>T variant included average fat (P=0.001) and grade fat (P=0.007), which both decrease significantly when there was a T allele present. The TT and GT genotypes for traits average and grade fat show nearly 2 mm less fat than the GG genotype. Fat thickness is an important trait when estimating composition of the carcass or yield grade (May *et al.* 2000; Williams 2002). Previous research by Cianzio *et al.* (1982) indicated a high association of total carcass fat and fat thickness between the 12^{th} and 13^{th} ribs. The effect observed with the GG genotype on the *HTR1B* variant on average and grade fat, would likely have an impact on yield grade, with potential to have positive implications for the producer and packer, as animals may be ready for slaughter at an earlier date.

Cutability or the per cent of lean yield on a beef carcass is a significant trait of importance. The *HTRIB* c.205G>T SNP was shown to have a significant association with cutability (P=0.001). The presence of a T allele(s) for cutability increases carcass yield by approximately 2%, over the GG genotype. Our data suggests that presence of the T allele or the TT genotype improves production and carcass traits associated with yield. While the allele frequency for the TT genotype is low, the GT genotype is moderate. Therefore selecting animals with the intention of improving yield traits such as cutability can be accomplished effectively. Polymorphisms found within the *HTR1B* gene in mice has previously shown to increase growth and feed intake (Sanders *et al.* 2002). It is possible that this is also an explanation for the

increased yield measurements observed in this study, where steers with a T allele are consuming more feed, resulting in a larger cutability percentage.

During carcass grading, REA is an essential component considered in determining the final grade. A trend was observed (P=0.061) between the *HTRIB* c.205G>T SNP and REA. The relatively large SEM (0.652) and low number of TT animals (n=21) likely explains the lack of significance. The TT and GT genotypes again had the larger REA measurements. This trait is important for determining the yield grade of the carcass and could result in a higher percentage of red meat, which would increase profitability.

It would be beneficial to study the *HTRIB* c.205G>T SNP in a larger population that would include in addition to the data already studied: marbling, days on feed and feed intake data to determine if this SNP could reduce time spent in the feedlot. Allele frequency for the TT genotype is low, therefore pooling genotypes for the TT and GT cattle may be beneficial for analysis, as the significant carcass traits demonstrate dominance of the T allele. This is beneficial for traits such as cutability and potentially REA, where the presence of a T allele improves carcass yield. Implementation of a test using the *HTRIB* c.205G>T variant would allow producers to more easily select and manage cattle according to their genotype, as they would only need to sort their cattle into two groups. They would be able to send and market their cattle based on either marbling using grid pricing or yield traits using live weight to obtain the best value for their beef cattle.

More research with a larger cattle population would be required to further evaluate the effects of the *HTRIB* c.205G>T variant on beef carcass traits. It would be beneficial to use camera grading to determine if *HTR1B* impacts the marbling score, as it does with average and

grade fat. Our knowledge about the bovine *HTR1B* gene is limited, so it would be beneficial to further explore its impact on animal performance and behavior in different cattle breeds.

A new significant association was found between the *HTRIB* c.205G>T SNP with average fat, grade fat and cutability. A trend was observed with carcass REA. There is mounting evidence that polymorphisms occurring in the HTR1B gene impact behaviors such as appetite and weight, found by researchers in mice (Sanders *et al.* 2002), BMI in women, (Levitan *et al*. 2001) and energy homeostasis (Zhang *et al.* 2008). Although finding significance in several economically important carcass traits in crossbred beef breeds is novel, validating the effects of the *HTRIB* c.205G>T SNP in a larger cattle population would be beneficial.

5.0 GENERAL DISCUSSION

There were three significant interactions occurring in the non-implanted steers: the *LEP c*.73C>T and *IGF2 c*. -292C>T for fat thickness, the *MC4R c. 856C>G* and *POMC c. 288C>T* for vision grade marbling score and the *GALR2 c. -199T>G* and *POMC c. 288C>T* interaction for REA. Fat thickness was found to be associated with TT/CT for *LEP* and TT/CT for *IGF2*, which led to a 1.83 mm increase compared to the smallest measurement. This interaction improves fat traits for *IGF2 c*. -292C>T and has potential for cattle to reach finishing stages earlier at an economic benefit to the producer (Goodall and Schmutz, 2007).

The TT/CT genotypes of *POMC* and the CC genotype from *MC4R* was found to be associated with vision grade marbling score, with a score of 472.54 which is a AAA carcass grade. Using this interaction as a DNA test may allow producers to target the grid pricing

marketing strategy, where the favorable allele combination could obtain premiums and minimize variation in pens sent for slaughter (Parish *et al.* 2009).

Favorable allele combinations for the final interaction includes TT for *POMC c. 288C>T* and GG for *GALR2* c. -199T>G, where there was a significant increase of 9.68 cm^2 in REA. The allele frequency for both alleles in *GALR2 c. -199T>G* are moderately heritable, but due to its significance for both yield and fat traits, this SNP could a good candidate for use as a DNA test. This could be a single gene DNA test or utilize the interaction with *POMC c. 288C>T*, while the T allele is rare, both CT and TT genotypes positively impact REA. Producers could select GG cattle for *GALR2 c. -199T>G* and TT/CT genotypes for *POMC c. 288C>T* to increase yield grade for a larger REA, or could select *GALR2* TG/TT cattle to improve fat traits.

Significant associations were identified between *HTRIB* c.205G>T SNP with carcass traits namely: average fat, grade fat and cutability and there was a trend with REA. Although finding significance with several economically important carcass traits in crossbred beef breeds is novel, validating the effects of the *HTRIB* c.205G>T SNP in a larger cattle population would be beneficial.

Many researchers will agree that gene interactions are not only complex, but also their analysis presents different challenges (Gilbert-Diamond and Moore, 2011). Through the discovery of SNPs associated with economically important carcass traits, we can use genomic selection, which will increase accuracy and minimize variability between animals when they are sent to market. It is clear that based on results from chapter three that gene interactions are complex, and many factors influence important phenotypes. However, large interaction networks have many benefits, and may allow us to improve predictions made for genetic selection and

increase our knowledge pool. The EPISNP networks identify associations between gene variants and carcass traits, so this can be used with our existing knowledge of molecular pathways and biology to further our understanding of quantitative traits and their underlying genetic mechanisms. However, from a producer or industry standpoint, it may be more beneficial to use MAM with single gene tests instead of interactions that contain multiple genes, which would be more beneficial when it comes to managing and sorting animals.

5.1. Future Research

Future research on gene interactions should assess genes with moderate and relatively equal allele frequencies to prevent high standard errors that come with multiple genotype combinations that include a small subset of animals, if they are to be used in a commercial setting. This would identify useful interactions and also accurately estimate the effect of the interaction, which will improve our ability to genetically select cattle based on their improved carcass traits.

The significant association found between the *HTRIB* c.205G>T SNP with average fat, grade fat and cutability warrants validation in a larger cattle population. Evidence indicates that the SNP impacts cattle performance, therefore future research should focus on other economically important carcass traits and its role in the appetite pathway. It may also be beneficial to pool dominant alleles for analysis of *HTRIB* c.205G>T to account for low frequency of the T allele.

6.0 LITERATURE CITED

Albrecht E., Teuscher, F., Ender, K., Wegner, J. 2006. Growth and breed related changes of marbling characteristics in cattle. J. Anim. Sci. **84(5)**: 1067-1075.

Ali, A.T., Hochfeld, W.E., Myburgh, R., Pepper, M.S. 2013. Adipocyte and adipogenesis. Eur. J. Cell Biol. 92:229-236.

Anderson, O.S., Sant, K.E. and Dolinoy, D.C. 2012. Nutrition and epigenetics: an interplay of dietary methyl donors, one-carbon metabolism and DNA methylation. J. Nut. Biochem. **23(8):** 853-859.

Anton, I., Kovacs, K., Hollo, G., Farkas, V., Lehel, L., Hajda, Z., Zsolnai, A. 2011. Effect of leptin, DGAT1 and TG gene polymorphisms on the intramuscular fat of Angus cattle in Hungary. Livestock Science. **135(3)**: 300-303.

Barendse, W., Vaiman, D., Kemp, S., Sugimoto, Y., Armitage, S., Williams, J., Sun, H., Eggen, A., Agaba, M., Aleyasin, S., Band, M., Bishop, M., Buitkamp, J., Byrne, K., Collins, F., Cooper, L., Coppettiers, W., Denys, B., Drinkwater, R., Easterday, K., Elduque, C., Ennis, S., Erhardt, G., Ferretti, L., Flavin, N., Gao, Q., Georges, M., Gurung, R., Harlizius, B., Hawkins, G., Hetzel, J., Hirano, T., Hulme, D., Jorgensen, C., Kessler, M., Kirkpatrick, B., Konfortov, B., Kostia, S., Kuhn, C., Lenstra, J., Leveziel, H., Lewin, H., Leyhe, B., Lil, L., Burriel, I., McGraw, R., Miller, J., Moody, D., Moore, S., Nakane, S., Nijman, I., Olsaker, I., Pomp, D., Rando, A., Ron, M., Shalom, A., Teale, A., Thieven, U., Urquhart, B., Vage, D., Weghe, A., Varvio, S., Velmala, R., Vilkki, J., Weikard, R., Woodside, C., Womack, J., Zanotti, M.,

Zaragoza, P. 1997. A medium-density genetic linkage map of the bovine genome. Mamm. Genome. **8:** 21–28.

Baldinger, P., Kraus, C., Rami-Mark, C., Gryglewski, G., Kranz, G.S., Haeusler, D., Hahn, A., Spies, M., Wadsak, W., Mitterhauser, M., Rujescu, D., Kasper, S., and Lanzenberger, R. 2015. Interaction between 5-HTTLPR and 5-HT1B genotype status enhances cerebral 5-HT1A receptor binding. NeuroImage. **111:** 505-512.

Becker, C., Riedmaier, I., Reiter, M., Tichopad, A., Pfaffl, M.W., Meyer, H.H.D. 2010. Effect of trenbolone acetate plus estradiol on transcriptional regulation of metabolism pathways in bovine liver. Horm. Mol. Biol. Clin. Investig. **2(2):** 257-65.

Bennett, G.L., Shackelford, S.D., Wheeler, T.L., King, D.A., Casas, E., Smith, T.P.L. 2013. Selection for genetic markers in beef cattle reveals complex associations of thyroglobulin and casein1-s1 with carcass and meat traits. J Anim Sci. 91**(2):** 565-571.

Benoit, S., Schwartz, M., Baskin, D., Woods, S.C. and Seeley, R.J. 2000. CNS melanocortin system involvement in the regulation of food intake. Horm. Behav. **37:**299-305.

Berga, L., Imholza, S., Versteega, S.A., Leegwatera, P.A.J., Zijlstrab. C., Bosmac, A.A., and Oostc, B.A. 2004. Isolation and characterization of the canine serotonin receptor 1B gene (htr1B). Gene. **326**: 131-139.

Bouwknecht, J.A., Gugten, J., Hijzen, T.H., Maes, R.A., Hen, R., and Olivier, B. 2001. Male and Female 5-HT (1B) receptor knockout mice have higher body weights than wildtypes. Physiol. Behav. **74:** 507-516.

Bruinvels, A.T., Landwehrmeyer, B., Gustafson, E.L., Durkin, M.M., Mengod, G., Branchek, T.A., Hoyer, D., and Palacios, J.M. 1993. Localization of 5-*HT1B,* 5-*HT1Dα,* 5-*HT1E* and 5-*HT1F* Receptor Messenger RNA in rodent and primate brain. Neuropharmacology. **33 (3/4):** 367-386.

Buchanan F.C., Thue T.D., Winkelman-Sim D.C., Plante Y. & Schmutz S.M. 2000. Two QTLs for growth map to bovine chromosome 14. In: 27th International Conference on Animal Genetics. July 22–26, Minneapolis, MN.

Buchanan, F.C., Fitzsimmons, C.J., Van Kessel, A.G., Thue, T.D., Winkelman-Sim, D.C., and Schmutz, S.M. 2002. Association of a missense mutation in the bovine leptin Gene with Carcass Fat Contents and Leptin mRNA. Gen. Sel. Evol. **43***:* 1- 12.

Buchanan, F.C., Thue, T.D., Yu, P., and Winkelman-Sim, D.C. 2005. Single nucleotide polymorphisms in the corticotrophin-releasing hormone and proopiomelanocortin genes are associated with growth and carcass yield in beef cattle. Animal Genetics. **36**: 127-131.

Canadian Beef Grading Agency. 2016. [Online] Available: http://www.beefgradingagency.ca/ [2015 June 03].

Casas, E., Shackelford, S.D., Keele, J.W., Stone, R.T., Kappes, S.M.; Koohmaraie, M. 2000. Quantitative trait loci affecting growth and carcass composition of cattle segregating alternate forms of myostatin. J. Anim. Sci. **78:** 560–569.

Cianzio, D. S., Topel D. G., Whitehurst, G. B., Beitz, D. C. and Self H. L. 1982. Adipose tissue growth in cattle representing two frame sizes: Distribution among depots. J. Anim. Sci. **55**:305.

Cianzio, D. S., D. G. Topel, G. B. Whitehurst, D. C. Beitz, and H.L. Self. 1985. Adipose tissue growth and cellularity: Changes in bovine adipocyte size and number. J. Anim. Sci. **60:**970–976.

Clark, D.L., Clark, D.I., Beever, J.E. And Dilger, A.C. 2014. Increased prenatal IGF2 expression due to the porcine IGF2 intron3-G3072A mutation may be responsible for increased muscle mass. J. Anim. Sci. **93(5):** 2546-2558.

Cockerham, C.C. 1954. An extension of the concept of partitioning hereditary variance for analysis of covariances among relatives when epistasis is present. Genetics. **39(6):** 859-882.

Corbin, C.H., O'Quinn, T.G., Garmyn, A.J., Legako, J.F., Hunt, M.R., Dinh, T.T.N., Rathmann, R.J., Brooks, J.C., Miller, M.F. 2014. Sensory evaluation of tender beef strip loin steaks of varying marbling levels and quality treatment. Meat Science. **100**: 21-31.

Dass, J.F.P. and Sudandiradoss, C. 2012. Insight into pattern of codon biasness and nucleotide base usage in serotonin receptor gene family from different mammalian species. Gene. **503(1):** 92-100.

Delavaud, C., Bocquier, F., Chilliard, Y., Keisler, Gertler, A. 2000. Plasma leptin determination in ruminants: effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. J. Endocrinol. **165:** 519-526.

Deobald, H.M., Buchanan, F.C. 2011. Characterization of two Pro-opiomelanocortin gene variants and their effects on carcass traits in beef cattle. BMC Genetics. **12** (2): 1471-2156.

Dering, C., Hemmelmann, C., Pugh, E., Ziegler, A. 2011. Statistical analysis of rare sequence variants: an overview of collapsing methods. Genet. Epidemiol., 35: S12–S17. doi: 10.1002/gepi.20643.

DiCostanzo, A. and Dahlen, C.R. 2000. Grid pricing as a fed cattle marketing strategy. Minnesota Cattle Feeder Report B-470. University of Minnesota, St. Paul, MN.

Du, X., Chen, C., Yuan, Z., Zhang, L., Chen, X., Wang, Y., Gao, X., Zhang, L., Gao, H., Li, J., and Xu, S. 2013. Genetic polymorphisms of Mc4R and IGF2 gene association with feed conversion efficiency traits in beef cattle. Pak. Vet. J. **33(4):** 418-422.

Du, F., Yin, L., Shi, M., Cheng, H., Xu, X., Liu, Z., Zhang, G., Wu, Z., Feng, G., and Zhao, G. 2010. Involvement of microglial cells in infrasonic noise-induced stress via upregulated expression of corticotrophin releasing hormone type 1 receptor. Neuroscience. **167(3):** 909-919.

Dubreucq, S., Kambire, S., Conforzi, M., Metna-Laurent, M., Cannich, A., Soria-Gomez, E., Richard, E., Marsicano, G., and Chaouloff, F. 2012. Cannabinoid Type 1 receptors

located on single-minded 1-expressing neurons control emotional behaviours. Neuroscience. **204:** 230-244.

Dynan, W.S. and Tijan, R. 1983. The-promoter-specific transcription factor Sp1 binds to upstream sequences in the SV40 early promoter. Cell. **35(1):** 79-87.

Ehrhardt, R.A., Slepetis, R.M., Bell, A.W., and Bosclair, Y.R. 2001. Maternal leptin is elevated during pregnancy in sheep. Domest. Anim. Endocrinol. **21:** 85-96.

Fantuzzi, G. and Faggioni, R. 2000. Leptin in the regulation of immunity, inflammation, and hematopoiesis. J. Leukocyte Biol. **68:**437-446.

Feinberg, A.P. 2007. Phenotypic plasticity and the epigenetics of human disease. Nature. **447:** 433-440.

Fisher, R.A. 1918. The correlation between relatives on the supposition of Mendelian inheritance. Trans.Roy. Soc. Edinburgh. **52:**399-433.

Forbes, S., Bui, S., Robinson, B.R., Hochgeschwender, U., Brennan, M.B. 2001. Integrated control of appetite and fat metabolism by the leptin-proopiomelanocortin pathway. Proc. Natl. Acad. Sci. **98 (7):** 7233-4237.

Gao, Y., Zhang, R., Hu, X., Li, N. 2007. Application of genomic technologies to the improvement of meat quality of farm animals. Meat Science. **77**(1): 36-45.

Gilbert-Diamond, D. and Moore, J.H. 2011. Analysis of gene-gene interactions. Curr. Protoc. Hum. Genet. **70(1):** 1.14:1.14.1-1.14.12.

Goodall, J.J. and Schmutz, S.M. 2003. Linkage mapping of IGF2 on cattle chromosome 29. Animal Genetics. **34**: 302-318.

Goodall, J.J. and Schmutz, S.M. 2007. IGF2 gene characterization and association with rib eye area in beef cattle. Animal Genetics. **38**: 154-161.

Gorocica-Buenfil, M.A., Fluharty, F.L., Bohn, T., Schwartz, S.J. and Loerch, S.C. 2007. Effect of low vitamin A diets with high-moisture or dry corn on marbling and adipose tissue fatty acid composition of beef steers. J. Anim. Sci. **85:**3355–3366.

Houseknecht, K.L., Baile, C.A., Matteri, R.L., Spurlock, M.E. 1998. The biology of leptin: A Review. J. Anim. Sci. **76:** 1405-1420.

Houston R.D., Cameron N.D., Rance K.A. 2004. A *melanocortin-4 receptor* (*MC4R*) polymorphism is associated with performance traits in divergently selected large white pig populations. Animal Genetics. **35:** 386–390.

Hosmer, D.W. and Lemeshow S. 2000. Applied Logistic Regression. John Wiley & Sons, New York.

Huang, M., Gao, X., Li, J.Y., Ren, H.Y., Chen, J.B., Xu, S.Z. 2010. Polymorphisms in *MC4R* gene and correlations with economic traits in cattle. Mol. Biol. Rep. **37(3):** 941-3944.

Ickowicz, A., Feng, Y., Wigg. K., Quist, J., Pathare, T., Roberts, W., Malone, M., Schachar, R., Tannock, R., Kennedy, J.L., Barr, C.L. 2007. The serotonin receptor HTR1B: gene polymorphisms in attention deficit hyperactivity disorder. Am. J. Med. Genet. B. Neuropsychiatr. Genet. **144B(1):** 121-125.

Ingvartsen, K.L. and Boisclair, Y.R. 2001. Leptin and the regulation of food intake, energy homeostasis and immunity with special focus on periparturient ruminants. Domest. Anim. Endocrinol. **21:** 215-250.

Kalra S.P., Dube M.G., Pu S., Xu B., Horvath T.L, Kalra PS. 1999. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocr. Rev. **20:**68–100.

Kaminsky, Z., Wang, S.C. and Petronis, A. 2006. Complex disease, gender and epigenetics. Annals of Medicine. **38(8):** 530-544.

Kempthorne, O. 1954. The correlation between relatives in a random mating population. Proc. R. Soc. Lond. B. Biol. Sci. **143:**102-113.

Killinger KM, Calkins CR, Umbergert WJ, Feuz DM and Eskridge KM 2004. Consumer sensory acceptance and value for beef steaks of similar tenderness but differing marbling level. J. Anim. Sci. **82:** 3294-3301.

King, D.A., Shackelford, S.D., McDaneld, T.G., Kuehn, L.A., Kemp, C.M., Smith, T.P.L., Wheeler, T.L., and Koohmaraie, M. 2012. Associations of genetic markers in cattle receiving differing implant protocols. J. Anim. Sci. **90:** 2410-2423.

Kononoff P.J., Deobald H.M., Stewart E.L., Laycock A.D. & Marquess F.L.S. 2005. The effect of a leptin single nucleotide polymorphism on quality grade, yield grade, and carcass weight of beef cattle. J. Anim. Sci. **83:** 927–32.

Kononoff, P. J., P. J. DeFoor, M. Engler, S. Swingle, S. T. James, H.M. Deobald, J. Deobald, and F. L. S. Marquess. 2013. Impact of a leptin single nucleotide

polymorphism and zilpaterol hydrochloride on growth and carcass characteristics in finishing steers. J. Anim. Sci. **91:**5011–5017.

Laporta, J., and Hernandez, L.L. 2015. Serotonin receptor expression is dynamic in the liver during the transition period in Holstein dairy cows. Domest. Anim. Endocrinol. **51**: 65-73.

Lang R., Gundlach A.L., Kofler B. 2007. The galanin peptide family: receptor pharmacology, pleiotropic biological actions, and implications in health and disease. Pharmacol. Ther. **115:**177-207.

Lefterova, M., and Lazar, M. 2009. New developments in Adipogenesis. Trends in Endocrinol. and Metabol**. 20(3):** 107-114.

Lerer, E., Kanyas, K., Karni, O., Ebstein, R.P., and Lerer, B. 2006. Why do young women smoke? II. Role of traumatic life experience, psychological characteristics and serotonergic genes. Mol. Psychiatry. **11(8):** 771-781.

Levitan, R.D., Kaplan, A.S., Masellis, M., Basile, V.S., Walker, M.L., Lipson, N., Siegel, G.I., Woodside, D.B., Macciardi, F.M., Kennedy, S.H., and Kennedy, J.L. 2001. Polymorphism of the serotonin 5-HT1B receptor gene (HTR1B) associated with minimum lifetime body mass index in women with bulimia nervosa. Biol. Psychiatry. **50(8)**: 640-643.

Lewontin R.C. 1992. Genotype and phenotype. in Keywords in evolutionary biology, eds Keller E., Lloyd E.A.(Harvard University Press, Cambridge, MA). 137–144

Li, B., and Leal, S.M. 2008. Methods for detecting associations with rare variants for common diseases: Application to analysis of sequence data. Am. J. Hum. Genet. **83 (3)**: 311-321.

Lopez-Campos, O., Aalhus, J.L., Okine, E.K., Baron, V.S., and Basarab, J.A. 2013. Effects of calf and yearling-fed beef production systems and growth promotants on production and profitability. Can. J. of Anim. Sci. **93(1):** 171-184.

Ma, L., Runesha, H.B., Dvorkin, D., Garbe, J.R., and Da, Y. 2008. Parallel and serial computing tools for testing single-locus and epistatic SNP effects of quantitative traits in genome-wide association studies. BMC Bioinformatics. **9:** 315 doi:10.1186/1471-2105-9-315.

Matias, I., Bisogno, T., and Marzo, V. Di. 2006. Endogenous cannabinoids in the brain and peripheral tissues: regulation of their levels and control of food intake. Int. J. Obesity. **30:** S7-S12.

May, S. G., N. S. Burney, J. J. Wilson, J. W. Savell, A. D. Herring, D. K. Lunt, J. F. Baker, J. O. Sanders, and S. B. Smith. 1995. Lipogenic activity of intramuscular and subcutaneous adipose tissues from steers produced by different generations of Angus sires. J. Anim. Sci. **73:**1310–1317.

May, S.G., Miles, W.L., Harris, J.J., Morgan, J.B., Garrett, R.P., Williams, F.L., Wise, J.W., Cross, H.R., and Savell, J.W. 2000. Using live estimates and ultrasound measurements to predict beef carcass cutability. J. Anim. Sci. **78:** 1255-1261.

McLean, K.L. and Schmutz, S.M. 2011. Melanocortin 4 receptor polymorphism is associated with carcass fat in beef cattle. Can. J. Anim. Sci. **91:** 75-79.

Mekli, K., Payton, A., Miyajima, F., Platt, H., Thomas, E., Downey, D., Lloyd-Williams, K., Chase, D., Toth, Z.G., Elliott, R., Ollier, W.E., Anderson, I.M., Deakin, J.F., Bagdy, G., and Juhasz, G. 2011. The HTR1A and HTR1B receptor genes influence stress-related information processing. European Neuropsychopharmacology. **21(1):** 129–139.

Miller, D.B., and O'Callaghan, J.P. 2002. Neuroendocrine aspects of the response to stress. Metabolism. **51(6):** 5-10.

Molotkov, A., X. Fan, and G. Duester. 2002. Excessive vitamin A toxicity in mice genetically deficient in ether alcohol dehydrogenase *Adh1* or *Adh3*. Eur. J. Biochem. **269:**2607–2612.

Montgomery, G.W., and Sise, J.A. 1990. Extraction of DNA from sheep white blood cells. New Zealand Journal of Agricultural Research. **33:3**, 437-441.

Morley, J.E. and Levine, A.S. 1982. Corticotropin releasing factor, grooming and ingestive behaviour. Life Sci. **31:** 1459- 1464.

Mulder, H.A., Bijma, P., and Hill, W.G. 2008. Selection for uniformity in livestock by exploiting genetic heterogeneity of residual variance. Gen. Sel. Evol. **40:** 37-59.

Nelson, M.R., Kardia, S.L.R., Ferrell, R.E., and Sing, C.F. 2001. A combinatorial partitioning method to identify multilocus genotypic partitions that predict quantitative trait variation. Genome Res. **11(3):** 458-470.

Nichols, B.M., Reuter, R.R., Cook, B.J. 2014. Evaluation of using half-sibling beef cows to increase growth and carcass uniformity of calf crops. The Prof. Anim. Sci. **30(1):** 37-42.

Owens, F.N., Dubeski, P., and Hanson, C.F. 1993. Factors that alter the growth and development of ruminants. J. Anim. Sci. **71:** 3138-3150.

Oka, A., Maruo, Y., Miki, T., Yamasaki, T., and Saito, T. 1998. Influence of vitamin A on the quality of beef from the Tajima strain of Japanese Black cattle. Meat Sci. **48:** 159-167.

Panea, B., Alberti, P., Olleta, J.L., Campo, M.M., Ripoll, G., Altarriba, J., and Sanudo, C. 2011. Intrabreed variability and relationships for 41 carcass and meat traits in Pirenaica cattle. Span. J. of Ag. Res**. 6(4):** 546-558.

Parker, J.A. and Bloom, S.R. 2012. Hypothalmic neuropeptides and the regulation of appetite. Neuropharmacol. **63 (1):** 18-30.

Parish, J. A., J. D. Rhinehart, and J. D. Anderson. 2009. Marketing Fed Cattle. Mississippi State University, Extension Service.

Pickworth, C.L., Loerch, S.C., Velleman, S.G., Pate, J.L., Poole, D.H., and Fluharty. F.L. 2011. Adipogenic differentiation state-specific gene expression as related to bovine carcass adiposity. J. Anim. Sci. **89:** 355-366.

Platter, W.J., Tatum, J.D., Belk, K.E., Scanga, J.A., Smith, G.C. 2003. Effects of repetitive use of hormonal implants on beef carcass quality, tenderness, and consumer rating of beef palatability. J. Anim. Sci .**81(4):** 984-996.

Pritchard, L.E., Turnbull, A.V., White, A.J. 2002. Pro-opiomelanocortin processing in the hypothalamus: impact on melanocortin signaling and obesity. J. Endocrinol. **172(3):** 411-421.

Prause, A., Guionaud, C.T., Klulowska-Rotzler, J., Giulotto, E., Magnani, E., Chowdhary, B.P., Philipp, U.T., and Mevissen, M. 2007. Chromosomal assignment of five equine *HTR* genes by FISH and RH mapping. Animal Genetics. **38:** 81-91.

Pugh, K.A., Stookey, J.M., and Buchanan, F.C. 2011. An evaluation of corticotrophin-releasing hormone and leptin SNPs relative to cattle behaviour. Can. J. Anim. Sci. **91**: 1-6.

Reik, W. and Walter, J. 2001. Genomic imprinting: parental influence on the genome. Nat. Rev. Genet. 2: 21.32.

Reinhardt C. 2007. Growth-promotant implants: Managing the tools. Vet. Clin. N. Am. Food A. **23:** 309-319.

Roche P.J., Crawford R.J., Fernley R.T., Tregear G.W. & Coghlan J.P. 1988. Nucleotide sequence of the gene coding for ovine corticotropin-releasing factor and regulation of its mRNA levels by glucocorticoids. Gene. **71:** 421–31.

Rocha, B.A., Scearce-Levie, K., Lucas, J.J., Hiroi, N., Castanon, N., Crabbe, J.C., Nestler, E.J., and Hen, R. 1998. Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. Nature. **393(6681)**: 175-178.

Rosenthal, M.J. and Morley, J.E. 1989. Corticotropin releasing factor (CRF) and age-related differences in behavior of mice. Neurobiol. Aging. **10:** 167-171.

Sanders, A.R., Duan, J., and Gejman, P.V. 2002. DNA variation and pychopharmacology of the human serotonin receptor 1B (*HTR1B*) gene. Pharmacogenomics. **3:** 745-762.

Saudou, F., Amara, D.A., Dierich, A., LeMeur, M., Ramboz, S., Segu, L., and Buhot, M.C. 1994. Enhanced Aggressive Pound-Maker in Mice lacking 5-HT1B Receptor. Science. **265:** 1875-1878.

Savli, M., Bauer, A., Mitterhauser, M., Ding, Y., Hahn, A., Kroll, T., Neumeister, A., Haeusler, D., Ungersboeck, J., Henry, S., Isfahani, S.S., Rattay, F., Wasak, W., Kasper, S., Lanzenberger, R. 2012. Normative database of the serotonergic system in healthy subjects using multi-tracer PET. NeuroImage. **63:** 447-459.

Sherman, E.L., Nkrumah, J.D., Murdoch, B.M., Li, C., Wang, Z., Fu, A., and Moore, S.S. 2008. Polymorphisms and haplotypes in the bovine neuropeptide Y, growth hormone receptor, ghrelin, insulin-like growth factor 2, and uncoupling proteins 2 and 3 genes and their associations with measures of growth, performance, feed efficiency, and carcass merit in beef cattle. J. Anim. Sc. **86**: 1-16.

Schmutz, S.M., Stookey, J.M., Winkelman-Sim, D.C., Waltz, C.S., Plante, Y., and Buchanan, F.C. 2001. A QTL study of cattle behavioral traits in embryo transfer families. J. of Hered. **92(3):** 290-292.

Slominski, A., Wortsman, J., Luger, T., Paus, R., Solomon, S. 2000. Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress. Physiol Rev. **80:**979–1020.

Snelling, W.M., Cushman, R.A., Keele, J.W., Maltecca, C., Thomas, M.G., Fortes, M.R.S., and Reverter, A. 2013. Breeding and Genetics Symposium: Networks and pathways to guide genomic selection. J. Anim. Sci. **91:** 537-552.

Templeton AR. 2000. Epistasis and complex traits. In: Wade M, Brodie III B, Wolf J, editors. Epistasis and the evolutionary process. Oxford: Oxford University Press.

Tronstad, R., Unterschultz, J. 2005. Looking beyond valued-based pricing of beef in North America. Supply Chain Manag.. **10**: 214-222.

United States Department of Agriculture. 2016. United States standards for grades of carcass beef. United States Department of Agriculture. Agricultural Marketing Service. Washington, DC.

Untergrasser, A., Cutcutache, I., Koressaar, T., Ye. J., Faircloth, B. C., Remm, M. and Rozen, S. G. 2012. Primer3- new capabilities and interfaces. Nucleic Acids Res. **40(15):** e115.

Van Eenennaam, A.L. and Drake, D.J. 2012. Where in the beef-cattle supply chain might DNA tests generate value? Anim. Prod. Sci. **52:** 185-196.

Voisinet, B.D., Grandin T., Tatum, J.D., O'Conner, S.F., Struthers, J.J. 1997. Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. J. Anim. Sci. **75**: 892-896.

Walker, J.J., Spiga, F., Waite, E., Zhao, Z., Kershaw, Y., Terry, J.R., and Lightman, S.L. 2012. The origin of glucocorticoid hormone oscillations. PLoS Biol. **10(6):** e1001341.

Ward, A.K., McKinnon, J.J., Hendrick, S., and Buchanan, F.C. 2012. The impact of vitamin A restriction and ADH1C genotype on marbling in feedlot steers. J. Anim. Sci. **90***:* 2476-2483.

Wileman, B.W., D.U. Thomson, C.D. Reinhardt, and Renter, D.G. 2009. Analysis of Modern Technologies Commonly Used in Beef Cattle Production: Conventional Beef Production Versus Nonconventional Production Using Meta-analysis. J. Anim. Sci. **87:**3418-26.

Williams, A.R. 2002. Ultrasound applications in beef cattle carcass research and management. J. Anim. Sci. **80(E. Suppl. 2):** E183-E188.

Wheeler, T.L., Cundiff, L.V., and Koch, R.M. 1994. Effect of marbling degree on beef palatability in *Bos Taurus* and *Bos indicus* cattle. J. Anim. Sci. **72**: 3145-3151.

Woronuk, G.N., Marquess, F.L., James, S.T., Plamer, J., Berryere, T., Deobald, H., Howie, S., and Kononoff, P.J. 2012. Association of leptin genotypes with beef cattle characteristics. Animal Genetics. **43**: 608-610.

Young, J.I., Otero, V., Cerdan, M.G., Falzone, T.L., Chan, E.C., Low, M.J., and Rubinstein, M. 1998. Authentic cell-specific and developmentally regulated expression of pro-opiomelanocortin genomic fragments in hypothalamic and hindbrain neurons of transgenic mice. Soc. Neurosci. **17:** 6631-6640.

Zhang, C.L., Chen, H., Wang, Y.H., Zhang, R.F., Lan, X.Y., Lei, C.Z., Zhang, L., Zhang, A.L., and Hu, S.R. 2008. Serotonin receptor 1B (HTR1B) genotype associated with milk production traits in cattle. Vet. Sc. **85:** 265-268.

Zhang Y., Proenca R., Maffei M., Barone M., Leopold L., Friedman J.M. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature. **372:** 425-432.

Zifa, E., and Fillion, G. 1992. 5-Hydroxytryptamine receptors. Pharmacol. Rev. **44:** 401-458.

Ziouzenkova, O., Orasanu, G., Sharlach, M., Akiyama, T.E., Berger, J.P., Viereck, J., Hamilton, J.A., Tang, G., Dolnikowski, G.G., Vogel, S., Duester, G., and Plutzky, J. 2007. Retinaldehyde represses adipogenesis and diet-induced obesity. Nat. Med. **13:**695–702.

Appendix A

Non-implanted CFL Steers (n=1000)							
Carcass Trait	*GALR2*	*ADH1C*	*LEP*	*IGF2*	*POMC*	*MC4R*	*CRH4*
VGMARB	<.0001	0.60	0.01	0.30	0.87	0.00	0.35
REA (cm^2)	0.003	0.30	0.16	0.02	0.00	0.56	0.22
Fat Thickness (mm)	0.01	0.16	0.02	0.02	0.00	0.03	0.77
Implanted CFL Steers (n=1000)							
Carcass Trait	*GALR2*	*ADH1C*	*LEP*	*IGF2*	*POMC*	*MC4R*	*CRH4*
VGMARB	<.0001	0.94	0.04	0.01	0.56	0.07	0.02
REA (cm^2)	0.002	0.07	0.28	0.63	0.02	0.59	0.09
Fat Thickness (mm)	0.001	0.84	0.07	0.01	0.65	0.13	0.39

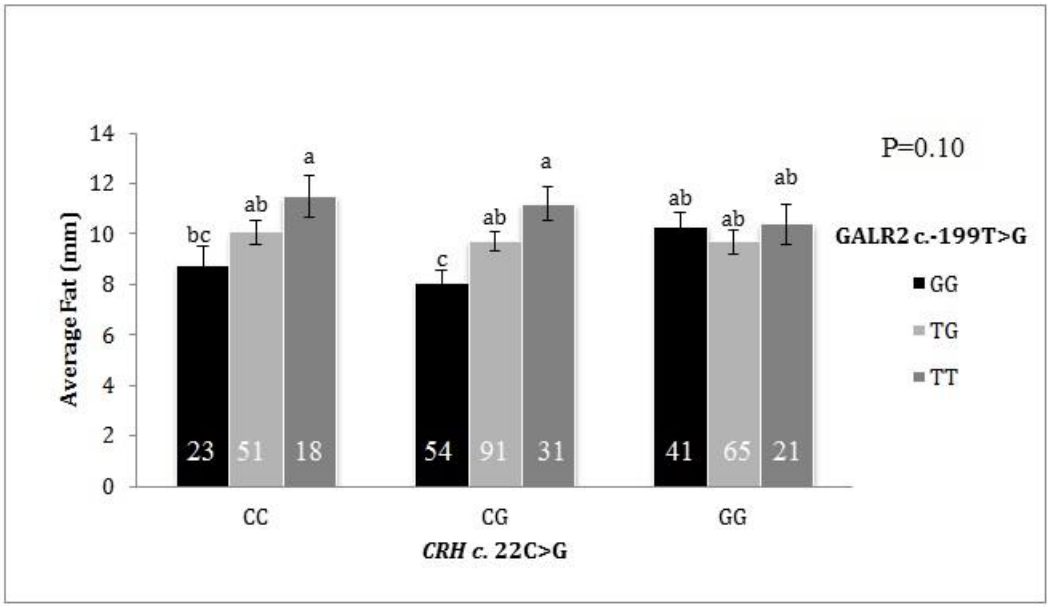
The MIXED procedure of SAS used for main effect p-values of carcass traits, where means were separated using the PDIFF statement.
VGMARB=Vision Grade Marbling Score; REA=Ribeye Area

Appendix B

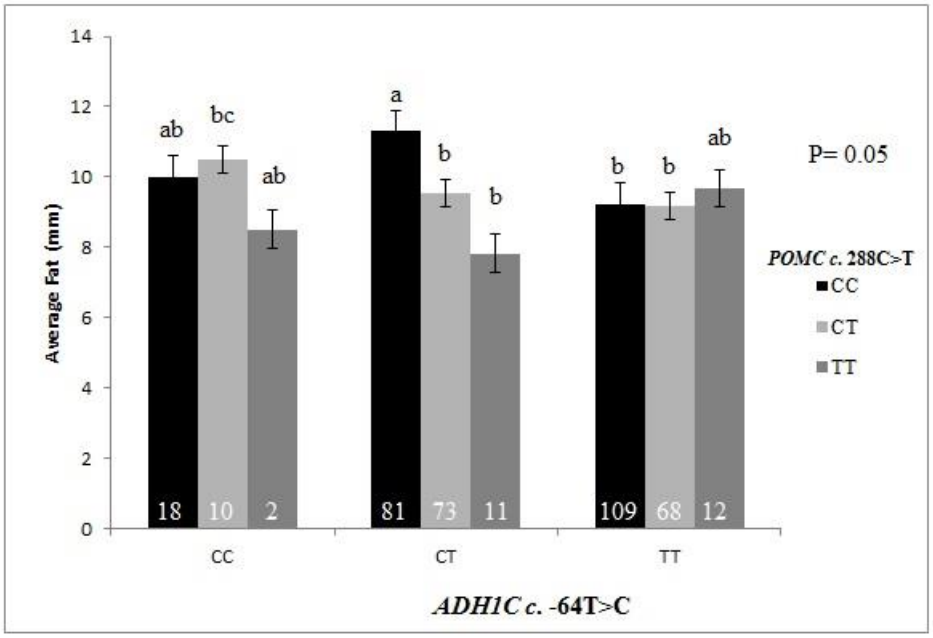
Carcass Trait	*GALR2 X CRH4*	*GALR2 X MC4R*	*GALR2 X POMC*	*GALR2 X IGF2*
Ave. Fat (mm)	0.10	0.29	0.01	0.46
Grade Fat (mm)	0.19	0.06	0.05	0.25
REA (cm^2)	0.33	0.03	0.34	0.23
Carcass Trait	*ADH1C X POMC*	*ADH1C X CRH4*	*ADH1C X MC4R*	*TG X POMC*
Ave. Fat (mm)	0.05	0.84	0.49	0.63
Grade Fat (mm)	0.03	0.63	0.37	0.63
REA (cm^2)	0.82	0.83	0.79	0.64
Carcass Trait	*TG X CRH4*	*TG X MC4R*	*LEP X IGF2*	*ADH1C X IGF2*
Ave. Fat (mm)	0.49	0.92	0.92	0.13
Grade Fat (mm)	0.64	0.92	0.79	0.10
REA (cm^2)	0.45	0.32	0.38	0.38
Carcass Trait	*TG X IGF2*	*LEP X POMC*	*LEP X CRH4*	*LEP X MC4R*
Ave. Fat (mm)	0.58	0.08	0.28	0.38
Grade Fat (mm)	0.43	0.06	0.48	0.32
REA (cm^2)	0.86	0.61	0.29	0.05
Carcass Trait	*LEP X CRH77*	*ADH1C X CRH77*	*TG X CRH77*	
Ave. Fat (mm)	0.30	0.80	0.84	
Grade Fat (mm)	0.38	0.46	0.95	
REA (cm^2)	0.05	0.61	0.53	

Pound-Maker gene variants P-Values with carcass data using the MIXED procedure of SAS
REA=Ribeye Area Ave. Fat=Average Fat

Appendix C



GALR2 c.-199T>G and *CRH c. 22C>G* interaction trend for Ave. Fat in the Pound-Maker steers. Animal numbers are in the bars and standard error is shown.



ADH1C c.-64T>C and POMC c. 288C>T interaction for Ave. Fat in the Pound-Maker steers. Animal numbers are in the bars and standard error is shown.

Appendix D

Top 13 most significant EPISNP Interaction mode results and P-Values for carcass traits in the Non-implanted Cattleland Feedyards Steers

EPISNP Non-implanted Cattleland Feedyards Steers				
Gene 1	**Gene 2**	**Carcass Trait**	**Interaction Mode**	**P-Value**
Leptin	*POMC*	REA (cm^2)	DD	0.004
Leptin	*POMC*	VGMARB	DA	0.07
POMC	*CRH*	Fat Thickness (mm)	AA	0.03
POMC	*CRH*	REA (cm^2)	AA	0.07
POMC	*GALR2*	VGMARB	AD	0.01
POMC	*GALR2*	REA (cm^2)	AD	0.06
CRH	*GALR2*	Fat Thickness (mm)	DA	0.00
CRH	*MC4R*	Fat Thickness (mm)	AA	0.05
ADH1C	*CRH*	Fat Thickness (mm)	DD	0.06
GALR2	*MC4R*	REA (cm^2)	DA	0.02
GALR2	*MC4R*	Fat Thickness (mm)	AA	0.04
MC4R	*IGF2*	VGMARB	AD	0.02
MC4R	*IGF2*	Fat Thickness (mm)	AD	0.03

Top 13 most significant EPISNP Interaction mode results and P-Values for the Implanted Cattleland Feedyards Steers

EPISNP Implanted Cattleland Feedyards Steers				
Gene 1	**Gene 2**	**Carcass Trait**	**Interaction Mode**	**P-Value**
ADH1C	*GALR2*	Fat Thickness (mm)	AD	0.09
ADH1C	*MC4R*	REA (cm^2)	DA	0.07
ADH1C	*MC4R*	VGMARB	AD	0.01
CRH4	*GALR2*	Fat Thickness (mm)	DD	0.08
CRH4	*MC4R*	VGMARB	DD	0.06
CRH4	*IGF2*	Fat Thickness (mm)	AD	0.03
GALR2	*IGF2*	REA (cm^2)	AA	0.04
Leptin	*MC4R*	VGMARB	AD	0.09
Leptin	*MC4R*	Fat Thickness (mm)	AA	0.09
MC4R	*IGF2*	VGMARB	DA	0.08
MC4R	*IGF2*	Fat Thickness (mm)	DD	0.06
POMC	*CRH4*	Fat Thickness (mm)	AD	0.07
POMC	*IGF2*	VGMARB	AA	0.01