CHARACTERIZATION OF PRO-OPIOMELANOCORTIN GENE VARIANTS AND THEIR EFFECT ON CARCASS TRAITS IN BEEF CATTLE

A Thesis Submitted to the College of
Graduate Studies and Research
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in the Department of Animal and Poultry Science
University of Saskatchewan
Saskatoon

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ABSTRACT

Pro-opiomelanocortin is a prohormone that codes for many different peptides, some of which are involved in the appetite pathway. A single nucleotide polymorphism c.288C>T in pro-opiomelanocortin (*POMC*) was previously demonstrated to be associated with hot carcass weight (HCW) and shipping weight (Ship wt) in cattle. While developing a commercial real time PCR test for the *POMC* c.288C>T we identified a 12 bp deletion *(POMC* c.293_304delTTGGGGGCGCGG). The deletion results in the removal of four amino acids; valine, two glycine, and alanine. The deletion does not cause a frame shift. Both the *POMC* c.288C>T SNP and the deletion were genotyped in 386 crossbred steers, and evaluated for associations with carcass traits. The animals with one copy of the deletion had a significantly lower end-of-background rib-eye area (P=0.04) and carcass rib-eye area (P=0.03) when compared to animals without the deletion. A significant association with the *POMC* c.288C>T SNP was found with start of finishing weight (SOF WT); (P=0.04), HCW (P=0.02), average fat and grade fat (P=0.05), carcass rib-eye area (REA); (P=0.03) and marbling (P=0.02). These results suggest that it would be beneficial for beef producers to know both the deletion and the *POMC* c.288C>T SNP genotypes when making marketing and culling decisions.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Fiona Buchanan for all of her guidance and encouragement. Thank you to my committee members Dr. Sheila Schmutz and Dr. Phyllis Shand, for their knowledge and giving me assistance in writing this thesis, as well as my chair person Dr. Bernard Laarveld. Thank you to my external examiner, Dr. Henry Soita.

Thank you to my fellow graduate students and friends Kim McLean, Chelsey Carruthers, Alison Ward and Kaley Pugh for all of their assistance. Thank you to my co-workers Tom Berryere, Steven James and Stacie Howie for their help and understanding while I was away from work.

To Leigh Marquess, thank you for providing me with the funding and opportunity to obtain my Masters. Since I have known you, you have allowed me to pursue my academic goals, encouraged me to stretch my limits, and consistently indicated your confidence and faith in my capabilities. You truly are a mentor and a friend.

Thanks you to my friends and family. To my mom, thank you for your unconditional support and encouragement. You have instilled in me the work ethic and confidence, which help me to succeed at whatever I choose to undertake. To my Grandpa, thank you for taking an interest and sharing your advice in our talks. Lastly, to my best friend and husband Jesse, thank you for your patience and support in my decision to pursue my Masters. You were always there to listen to my frustrations, keep me going and support me in every way possible.

DEDICATION

I would like to dedicate this thesis in memory of my dad Dwaine Deobald. He was an amazing person, whose invaluable teachings shaped me to be the person I am today. The values he instilled in me, and his high regard for further education, were my inspiration.

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LIST OF ABBREVIATIONS

ACTH	adrenocorticotropin
AGRP	agouti-related protein
αMSH	alpha melanocyte stimulating hormone
ANOVA	analysis of variance
ARC	arcuate nucleus
Arg	arginine
ADG	average daily gain
B-ADG	backgrounding average daily gain
Bp	base pair
β end	beta-endorphin
βLPH	beta lipotrophin
β-MSH	beta melanocyte stimulating hormone
CART	cocaine and amphetamine-regulated transcript
CRH	corticotropin-releasing hormone
DNA	deoxyribonucleic acid
DOF	days on feed
EOB BF	end of backgrounding back fat, measured by ultrasound
EOB REA	end of backgrounding rib-eye area, measured by ultrasound
F ADG	finishing ADG
HCW	hot carcass weight
HPA	hypothalamic-pituitary adrenal
γ-LPH	gamma lipotrophin
γ-MSH	gamma melanocyte stimulating hormone
Gly	glycine
JP	joining peptide
LSM	least-squared means
Leu	leucine
Lys	lysine
$MgCl_2$	magnesium chloride
MC1R	melanocortin 1 receptor
MC2R	melanocortin 2 receptor
MC3R	melanocortin 3 receptor
MC4R	melanocortin 4 receptor
MC5R	melanocortin 5 receptor
MCH	melanin-concentrating hormone
Tm	melting temperature
MMI	Metamorphix Inc
µl	microliter
mM	millimolar
MGV	molecular genetics value
NPY	neuropeptide Y
NT	n-terminal
PCR	polymerase chain reaction
pmol	picomole

POMC	pro-opiomelanocortin
QTL	quantitative trait loci
REA	rib-eye area
RFLP	restriction fragment length polymorphism
RT	reverse transcription
SER	serine
SNP(s)	single nucleotide polymorphism(s)
SD	standard deviation
SEM	standard error of the mean
SOB WT	start of backgrounding
SOF WT	start of finishing weight

1 INTRODUCTION

The cattle feeding industry has been in existence in Canada for many years. Typical breeding management practices involve evaluating expected progeny differences (EPDs) and visually scoring animals that would be selected for breeding purposes. In today's markets with close margins and little room for inefficiency, breeders and feedlots are turning to science, especially genetics, to help minimize cost and increase cattle production efficiency.

Genetic effects are partially responsible for differences in carcass composition among individual animals (Kononoff *et al.* 2005). One gene that has been shown to affect carcass traits in beef cattle is pro-opiomelanocortin (*POMC*). In 2003 Thue and Buchanan found the *POMC* c.288C>T single nucleotide polymorphism (SNP) and mapped it to chromosome 11. This area where *POMC* resides had also been identified as a region harbouring a quantitative trait locus (QTL) affecting carcass weight and average daily gain (Thue and Buchanan 2003). Buchanan *et al.* (2005) found that cattle homozygous for the thymine allele had an increased shipping weight of up to 9 kg per animal. The thymine allele was also associated with increased HCW and showed a trend with average daily gain (Buchanan *et al.* 2005).

Carcass composition is the main component used to value carcasses. The sex, breed, age and genetic background as well as nutrition all affect carcass composition (Bruns *et al*. 2004). Therefore it is important to study genes that are part of the appetite pathway that may ultimately affect carcass composition. Since an association had already been observed between a variant in *POMC* with hot carcass weight and shipping weight (Buchanan *et al.* 2005) it was important validate this association in another population. Associations were also evaluated on any new variants discovered in the *POMC* gene.

2 LITERATURE REVIEW

2.1. Appetite Pathway

The hypothalamus is the key area of the brain for appetite regulation. It plays an important role in the regulation of food intake and energy homeostasis. Energy homeostasis is not directly controlled by the hypothalamus, but rather is regulated by neuronal circuits through neuropeptides (Wynne *et al.* 2005). These neurons are located in the arcuate nucleus (ARC) of the hypothalamus (Kalra *et al.* 1999). Within the ARC there are two circuits that play an important role in both nutritional status and regulating energy homeostasis (Cone *et al.* 2001). One neuronal circuit contains the anorexigenic neuropeptides: pro-opiomelanocortin (POMC), cocaine-and amphetamine-regulated transcript (CART); (Elias *et al.* 1998; Kristensen *et al.* 1998) and corticotropin-releasing hormone (CRH); (Ingvartsen and Boisclair 2001). The other circuit contains orexigenic peptides consisting of neuropeptide Y (NPY) and agouti-related protein (AGRP) which stimulate food intake (Broberger *et al.* 1998; Hahn *et al.* 1998) as well as melanin-concentrating hormone (MCH) and galanin (Ingvartsen and Boisclair, 2001.)

Another important hormone involved in energy homeostasis is leptin. Leptin is produced by adipose tissue (Banks *et al.* 1996). Leptin's actions are carried out through its role in the activation of the anorexigenic circuit. This action is carried out due to the co-existence of neuropeptide and leptin receptors on neurons in the hypothalamus. Changes in the plasma concentration of leptin regulate the synthesis of these orexigenic and anorexigenic peptides (Wynne *et al.* 2005; Ingvartsen and Boisclair 2001). Houseknecht *et al.* (1998) explained in detail the role that leptin plays in energy expenditure, food intake, and whole-body energy balance. Some of these roles, in particular food intake, are implicated through *POMC* peptides. Cheung *et al.* (1997) showed that *POMC* expressing neurons are direct targets for leptin. *POMC* works to reduce appetite through the MSH peptides generated from it. The food intake reduction is due to the anorectic alpha MSH (αMSH) which reduces appetite when bound to melanocortin 3-receptor (MC3R) or melanocortin 4-receptor (MC4R). An antagonist of MC3R and MC4R is the previously mentioned hypothalamic neuropeptide AGRP (Haskell-Luevano *et al.* 1999).

2.2. Pro-opiomelanocortin

Pro-opiomelanocortin is expressed in several mammalian tissues including the arcuate nucleus of the hypothalamus, the anterior and intermediate lobes of the pituitary, skin, testis and immune system (Pintar *et al.* 1984; Bertagna 1994; Castro *et al.* 1997; Young *et al*. 1998). *POMC* is a precursor protein that encodes for numerous bioactive peptides known as the melanocortin peptides. These peptides include adrenocorticotrophin (ACTH), alpha melanocyte stimulating hormone (α-MSH), beta melanocyte stimulating hormone (β-MSH), and gamma melanocyte stimulating hormone (γ-MSH); (Eipper and Mains 1980). The *POMC* prohormone also generates the opiate peptide β-endorphin (β-END); (Gantz and Fong 2003). This is where the "opio" section comes from in pro-opiomelanocortin. Pritchard *et al.* (2002) depicts a clear diagram showing the various peptides that are cleaved from *POMC* (Figure 2.1). The biological effects of the *POMC* peptides are mediated through the binding of these peptides to specific receptors (Pritchard *et al.* 2002).

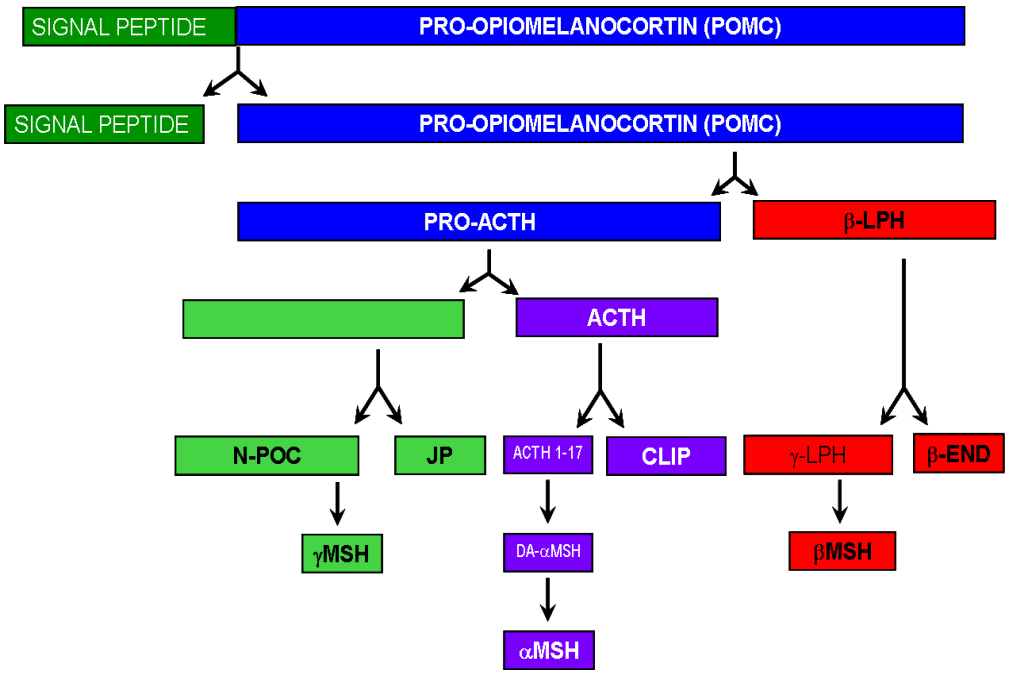

Figure 2.1 The *POMC* processing pathway in the hypothalamus. The precursor protein includes three main regions: adrenocorticotrophin (ACTH) which is cleaved to produce alpha melanocyte stimulating hormone (α-MSH), β-lipotrophin (β-LPH) that is later cleaved to γ-lipotrophin (γ-LPH), beta endorphin (β-END) and then β-MSH, and the N-terminal gamma melanocyte stimulating hormone (γ-MSH); (Pritchard *et al.* 2002) with permission.

2.2.1. Gene Structure

An alignment of human, cattle, pig and monkey *POMC* cDNA sequences revealed 85% homology among the species (Can *et al.* 1998). In an earlier study by Whitfeld *et al.* (1982), the specific region containing ACTH was 95% homologous between the human and bovine sequence.

In humans the *POMC* gene maps to chromosome 2p23.3 (Satoh and Mori 1997), and in cattle to chromosome 11 (Thue and Buchanan 2003). The first reported nucleotide sequence of the complementary DNA (cDNA) for bovine *POMC* was published by Nakanishi *et al.* (1979). Sequencing of the *POMC* gene in mammalian species has confirmed the gene consists of three exons and two introns (Millington, 2007). The gene itself produces a propeptide with a mass of 32 kilodaltons (kDa). The human *POMC* gene is 7665 base pairs (bp) long, while in cattle the gene is 7493 bp long. The actual coding region begins in exon 2 and ends in exon 3 and is 792 bp (Figure 2.2).

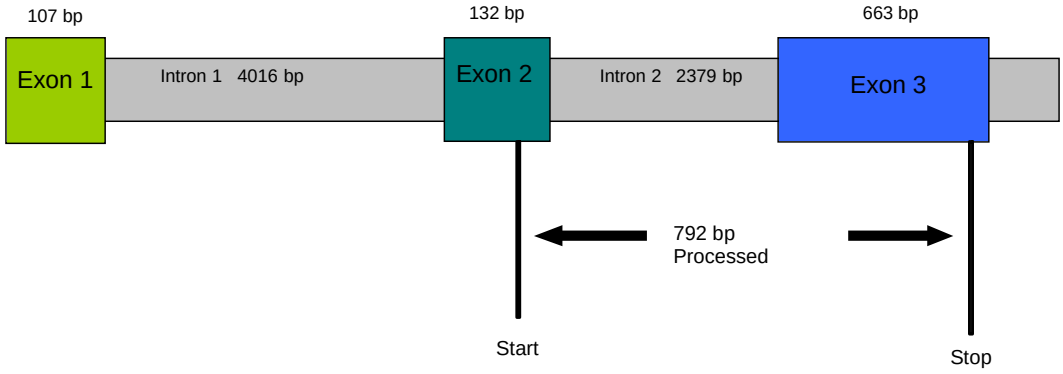

Figure 2.2 Gene structure of bovine *POMC*

2.2.2. Gene Processing

In order to generate the numerous peptides produced from POMC there are many post-translational cleavages that must occur. In the early stages the single protein that is formed from the mRNA is passed through the golgi stacks. The signal peptide binds to a sorting receptor called carboxypeptidase E (CPE) and is directed to the regulated secretory pathway (RSP); (Cool *et al.* 1997). Within RSP granules POMC is post-translationaly cleaved by prohormone

convertases (PC1 and PC2) to produce its range of bioactive peptides (reviewed in Pritchard *et al.* 2002).

The prohormone convertases (PC) potentially target the eight pairs, and one quadruplet of basic amino acids contained within POMC. The peptides that are cleaved as a result of the PCs are dependent on the cleavage specificity of the tissue (Figure 2.3).

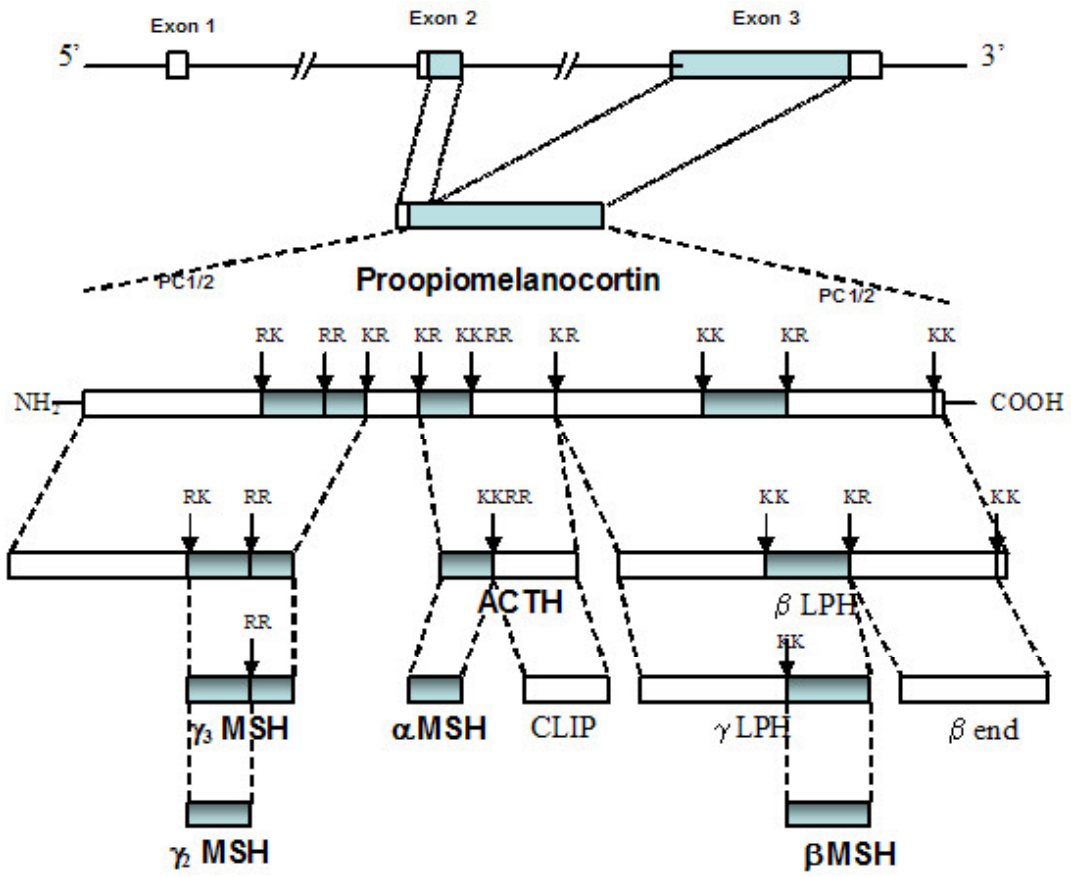

Figure 2.3 Gene structure and post-translational processing of pro-opiomelanocortin (POMC). Prohormone convertases 1 and 2 (PC1/2) cleave the *POMC* peptides at dibasic amino acid residues consisting of lysine (K) and arginine (R). The peptides produced include: adrenocorticotrophin (ACTH), the N-terminal gamma melanocyte stimulating hormone (γ-MSH), alpha melanocyte stimulating hormone (α-MSH); β-lipotrophin (β-LPH), γ-lipotrophin (γ-LPH), β melanocyte stimulating hormone (β-MSH) and beta endorphin (β-END) (Millington 2007) with permission.

PC1 is only present in the corticotroph cells. There are four cleavage sites located within the corticotroph cells of the anterior pituitary. These cleavage sites are all lysine-arginine (Lys-Arg). The peptides that are generated in the corticotroph cells are: N-terminal (NT), joining peptide (JP), adrenocorticotrophin hormone and beta lipotrophin (Figure 2.4). A small amount of gamma lipotrophin (γ-LPH) and beta-endorphin (β-END) are generated, but are used minimally by molecules (Bertagna 1994; Raffin-Sanson *et al.* 2003).

In the melanotroph cells, which are located in the intermediate lobe of the pituitary, all of the cleavage sites are utilized, as both PC1 and PC2 are expressed in this cell type. The additional peptides that are produced in the melanotroph cells are: γ^3MSH γ^2MSH, γ^1MSH, ACTH (which is further cleaved to produce α-MSH), and β-MSH (Zhou *et al.* 1993; Friedman *et al.* 1998). The C-terminal end of N-POMC is cleaved between Arg and Lys to generate γ^3MSH, which can be further cleaved to γ^2MSH and γ^1MSH. For the purpose of this thesis γ^3MSH will be known as γ-MSH as this is the most common form (Denef *et al*. 2003).

2.2.3. Role of *POMC* Peptides

The peptides produced from the prohormone POMC, α, β and γ-MSH and ACTH, are mediated by a family of five G-Protein-coupled receptors MC1R to MC5R (Yeo *et al.* 2000). There are a number of tissues that have these receptors. The biological functions that occur once the receptor has bound a POMC peptide are dependent on the tissues in which the receptor is present (Harmer and Bicknell, 2005).

MC1R is expressed predominantly in the skin and binds α-MSH, which is important in determining skin and hair pigmentation (Gantz and Fong 2003). MC2R, which is expressed in the adrenal cortex zona reticularis and zona fasiculata, is limited to activation by ACTH (Gantz and Fong 2003). MC3R and MC4R are expressed in areas of the brain that have been shown to be involved in appetite and energy homeostasis (Gantz and Fong 2003). MC3R is expressed on *POMC* neurons of the arcuate nucleus, and γ-MSH is the only peptide with significant affinity to bind to it. The MC4R receptor is expressed in the brain and studies have confirmed that it is important in homeostasis and also plays a role in feeding behaviour (Hadley and Haskell-Luevano 1999). The peptide αMSH acts as a ligand for the MC4R which, when bound, diminishes appetite (Marsh *et al.* 1999). Poggioli (1986) also showed that injection of α-MSH into specific areas of the brain resulted in inhibition of food intake.

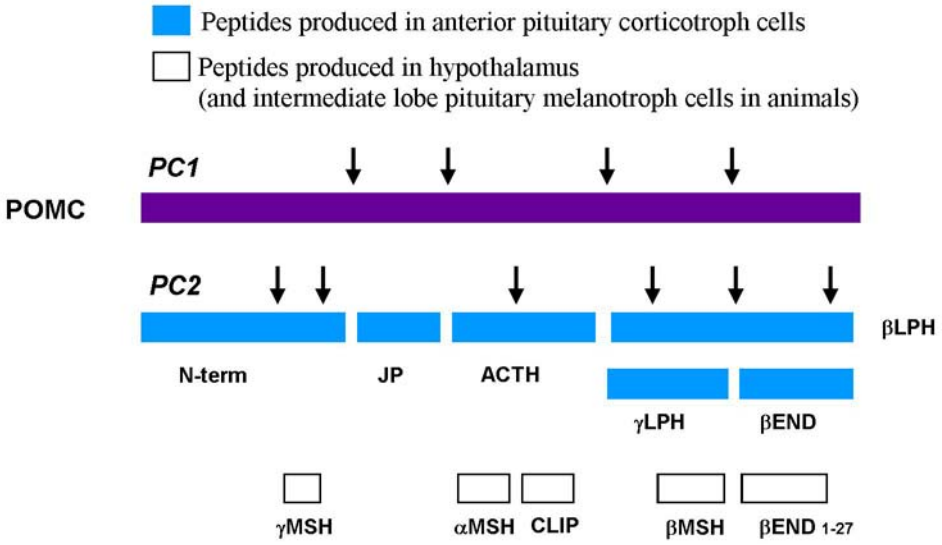

Figure 2.4 Location of prohormone convertases 1 and 2 (PC1/PC2) cleavage in pro-opiomelanocortin (*POMC*) processing (Raffin-Sanson et al. 2003) with permission.

In 1999, Marsh *et al.* used MC4R-deficient ($MC4R^{-/-}$) mice and found that when these mice were given an MSH-like agonist, the mice did not respond. This supported the study by Huszar *et al.* (1997) where mice that were lacking MC4R (homozygous mutant) ate 36% more than the controls. The mutant mice were also on average twice as heavy as the wild type mice at the end of 15 weeks. This further supported that when the MC4R receptor is ligated with α-MSH, it has anorectic effects. An antagonist of MC3R and MC4R is the hypothalamic neuropeptide agouti-related peptide (AGRP); (Haskell-Luevano *et al*. 1999). The other receptor MC5R is expressed in areas such as the adrenal gland and adipocytes (Chhajlani 1996). For the purpose of this thesis we are most interested in MC3R and MC4R because they are expressed in regions of the brain that are involved in feeding behaviour.

2.2.4. *POMC* Mutations

The importance of *POMC* in the appetite pathway makes it a strong candidate gene for any body weight trait. Thus researchers have been searching this gene for polymorphisms. In humans, Krude *et al.* (1998) found 3 mutations in the *POMC* gene. Two of these mutations were located in exon 3: G7013T that causes a premature stop codon, and C7133Δ which causes a

frame-shift and also introduces a premature stop codon. The third mutation C3804A, occurs in exon 2 and also results in premature stop codon. All three SNPs were found to be associated with obesity.

In a molecular screening by Giudice *et al.* (2001) on obese Italian children, three mutations resulting in non-conserved amino acid substitutions were discovered. The first mutation was G3834C, which results in a Ser with Thr substitution, the second was C3840T, resulting in a substitution of Ser with Leu. And lastly C7406G, which resulted in the substitution of Arg with Gly. All three mutations could potentially have contributed to the childrens' obesity.

Research into polymorphisms in *POMC* has not been limited to humans. In 2003, Thue and Buchanan discovered a SNP in the cattle *POMC* gene at base pair 288 (c.288C>T). This SNP does not alter the amino acid; it remains a serine. Further research on the *POMC* c.288C>T SNP, using 256 Charolais-cross steers, demonstrated an association with both shipping weight and hot carcass weight (Buchanan *et al.* 2005).

2.2.5. *POMC* Influence on Appetite and Obesity

The *POMC* derived peptides, mainly α, β and γ-MSH, target neurons that express the melanocortin receptors MC3R and MC4R which are located in areas of the brain that are known to control appetite. When these receptors bind one of the *POMC* peptides, the result is a decrease in food intake and an increase in energy expenditure (Yeo *et al.* 2000; Pritchard *et al*. 2002).

Krude *et al.* (1998) carried out a study linking *POMC* with obesity. In this study, patients with mutations in *POMC* showed early onset obesity, adrenal insufficiencies and red hair pigmentation. Krude *et al.* (1998) explained that in these patients there was likely a loss of the production of ACTH, α-MSH and β-MSH. Due to the absence of these peptides in the patients, MC4R was unable to bind αMSH and decrease appetite leading to obesity.

A recent study by Tung *et al.* (2006) used a mouse model deficient in *POMC* peptides (*POMC*$^{-/-}$). The mouse was then used to study the role of α-MSH, γ3MSH, γ2 MSH and γ lipotropin (γ-LPH) in energy homeostasis. The peptides were administered to the *POMC*$^{-/-}$ mice in equal amounts while they were feeding. After a three-day period, the results showed that α-

MSH was the only peptide to have a significant effect on body weight (reducing it). This in turn affected both lean mass and fat in the mouse. The remaining peptides all reduced food intake in the mice, but to a much lesser degree than α-MSH.

Fehm *et al.* (2001) used thirty-six normal weight humans to study the role of melancortins (MSH/ACTH) in fat stores. The humans were divided into three groups. The first group was treated with MSH/ACTH, and the second group received another agonist of the MC4 receptor, desacetyl-α-MSH, and the final group was given a placebo. The groups were given these treatments twice a day for six weeks. At the end of six weeks the group administered the MSH/ACTH had reduced body fat by an average of 1.68 kg and their body weight was 0.79 kg less. The group that was administered desacetyl-α-MSH had no significant change. This study further supports an anorexigenic effect of *POMC* peptides, which can contribute to lower body weights.

In a study performed by Yaswen *et al.* (1999), they genetically manipulated mice to be $POMC^{-/-}$ by replacing exon 3 with a neomycin-resistance cassette. Mice lacking the *POMC* peptides had weights twice that of mice with the *POMC* peptide region. They also concluded that mice lacking *POMC* derived peptides were not only obese but had defective adrenal development and displayed altered pigmentation.

In humans, Challis *et al.* (2002) described a missense mutation that disrupts the dibasic amino acid cleavage site between β-MSH and β-END. A meta-analysis study of this mutation in *POMC* gene in obese children suggested that this mutation led to a genetic predisposition to childhood obesity (Coll *et al.* 2004).

Along with the numerous studies looking at the role *POMC* peptides play in obesity, there are also studies looking at the role of the MC4 receptor. It is well known that the MC4R receptor is involved in the regulation of appetite and body weight in both humans and mice. Huszar *et al.* (1997) reported MC4R deficient mice showed hyperphagia, hyperinsulinemia, and hyperglycemia.

Kim *et al.* (2000) had similar findings when they identified a missense mutation in the porcine *MC4R* gene. They reported significant associations of specific genotypes, with backfat, growth rate and feed intake. Yeo *et al.* (1998) identified a frameshift mutation in MC4R caused

by a 4bp deletion in humans. This deletion resulted in obesity in both the father and child who were heterozygous for the deletion. This further supports the link between mutations in MC4R and human obesity.

2.3. Pseudogenes and Deletions

One of the first reports on pseudogenes was published by Jacq *et al.* (1977). They found a 5S rRNA-related gene that was almost an exact repeat of the functional gene. This newly isolated sequence was truncated by 16 base pairs at the 5' region and had 14 bp mismatches. They concluded this fragment could not be functional and termed it a "pseudogene".

Through many years of research a better understanding of pseudogenes exists, although there are still many unanswered questions. It is believed that scientists have now identified more than 19,000 pseudogenes (Gerstein and Zheng 2006).

There are two main types of pseudogenes, non-processed (duplicated) and processed pseudogenes. Non-processed pseudogenes arise after part or all of a gene segment is duplicated. This type of pseudogene is usually found in the area of the functioning gene (Harris *et al.* 1984). Non-processed genes may contain introns and flanking regulatory sequences, although there is usually loss of function due to mutations (Torrents *et al.* 2003; D'Errico *et al.* 2004). There are often other differences between the non-processed segment and the gene it is duplicated from; such as deletions, insertions, point mutations, premature stop codons and alterations in splice sites (D'Errico *et al.* 2004). Although a non-processed gene is usually non-functional, Prince and Picket (2002) believe that non-processed genes are the main reason for the existence of new gene function or expression profiles.

Processed pseudogenes, unlike non-processed pseudogenes, originate from mRNA (D'Errico *et al.* 2004). Their existence is due to a three-stage process. In the first step, the DNA from the gene is transcribed into an RNA transcript. The introns are then removed to produce mRNA. In the final stage, the mRNA that would normally be transcribed into a protein is instead reverse transcribed back into DNA and inserted back into a chromosome. Common characteristics of processed pseudogenes are: they lack an upstream regulatory region, they lack introns, and at the 3' end they have a poly A tail flanked by direct repeats. Another commonality of processed pseudogenes is that typically the reverse transcription (RT) process is terminated prematurely. Resulting in a 5' truncated copy of DNA lacking the promoter region, thus making

it a non-functional gene. Even if a processed pseudogene is created with a promoter region it could be inserted into a genomic region that is unacceptable for its expression, making it again nonfunctional (D'Errico *et al.* 2004).

Once a processed pseudogene is inserted back into a chromosome it may undergo one of two evolutionary processes (D'Errico *et al.* 2004). One of the processes is called 'compositional assimilation; whereby the pseudogene becomes susceptible to a high degree of point mutations that over time, degrade the similarity between the pseudogene and its functional counterpart. The second process involves the pseudogene having a reduction in size. This change is due to an increase in deletions rather than insertions (D'Errico *et al.* 2004). D'Errico *et al.* (2004) estimated that the latter of these processes occurs very slowly over time and it is estimated that it can take 400 million years for a processed pseudogene to lose half of its DNA.

2.4. Application of DNA testing in the Cattle Industry

In 2005, a study by the National Beef Quality Audit released 10 goals they thought would help give American beef a competitive advantage. Some of the key goals were to minimize the production of excess fat and to strive for uniformity and consistency within cattle production (Smith et al. 2005). Another important goal was to select management practices that increase value. Traditionally selection for more productive animals used methods such as EPDs and indexing. Now, thanks to research by animal geneticists, producers are realizing the benefits of looking at the genetic profiles of their animals to determine their economic potential. Today there are many companies offering various management practices and tools based on the genotype at one or a few genes to give producers the best returns on their cattle.

Merial, a large animal pharmaceutical company, uses a program called Igenity™. The Igenity™ program offers an IGENITY profile that allows users to identify some traits such as feed efficiency carcass traits, tenderness, docility, coat color, quality grade and marbling. Within each trait they have identified multiple markers or SNPs that are purported to be responsible for the phenotypic results. The markers are scored and averaged to generate a profile score for each trait, from a low of 1 to a high of 10. Producers can use these scores to make breeding and selection decisions depending on which animal production traits they are trying to optimize or achieve (Igenity 2009).

Metamorphix Inc (MMI), uses DNA testing to "improve the global food supply and human health" and in livestock their goal is to help customers "produce higher quality, nutritious meat more efficiently" (Metamorphix Inc. 2004). MMI, like Merial, utilizes numerous SNPs for a single trait to generate a number value, which they call the Molecular Genetics Value (MGV). Their scoring system differs by giving animals with an average MGV a score of 0; if an animal obtains a score above 0 it has a positive MGV and those below 0 have a negative MGV. Marbling is one of the traits that can be given an MGV score; this test is called Tru-Marbling™. Once a producer has had their herd tested for Tru-Marbling™ they can begin identifying and ranking their herd's genetic potential for marbling, which should correlate to the results from carcass grading at a packing plant (Metamorphix Inc. 2004).

Pfizer Animal Genetics, a division of Pfizer Animal Health, uses DNA tests under the name GeneSTAR®. These tests are used to analyze both quality and production traits in beef cattle. The initial test, in 2000, was a single marker identified in the thyroglobulin gene that influenced marbling. Since then they have added additional DNA tests that are associated with feed efficiency, marbling and tenderness. GeneSTAR® now uses Molecular Value Predictions (MVPs) to make breeding and management decisions, rather than their older method, which involved a rating system that used stars. To obtain the MVPs for feed efficiency, marbling and tenderness, 56 DNA markers are utilized. Once an animal has a MVP generated, breeding decisions can be made for you based on the MVPs within those traits that need improving (Pfizer 2009).

Another company using molecular genetics to create value in the cattle industry is Quantum Genetics Canada Inc (QGCI). Unlike the previous mentioned companies, QGCI currently uses only one SNP to help producers in their management decisions. The focus of their business is in the management of the cattle in feedlots. They offer a management tool called Quantum Management Protocol (QMP). This protocol involves the convergence of one SNP with an ultrasound scan to predict optimal days on feed (DOF) for feedlot animals. The trade name of the test is Quantum-L, and it is integrated into QMP. This SNP has been scientifically shown to be associated with slaughter subcutaneous fat levels (Buchanan *et al.* 2002; Kononoff *et al.* 2005). Using the Quantum-L test, QMP determines the optimal DOF for each animal resulting in a more uniform carcass that has reached its optimal carcass composition without being over or underfed. Consequently, producers can achieve optimal value for their animals

without putting excess feed into the animals while eliminating the risk of underfeeding. Purebred producers can use the Quantum-L test as a breeding tool to improve herd genetics and create a calf crop desired by feedlots.

2.5. Objective & Hypothesis

The objectives for this thesis were to characterize polymorphisms in *POMC* and determine traits that were significantly affected by any polymorphisms. Another objective was to design a commercial real time PCR test for the SNP in *POMC* c.288C>T. This test would then be used to genotype 386 crossbred steers for the *POMC* c.288C>T SNP and determine if there was an association with carcass traits in beef cattle. The purpose for this was to validate the results presented by Buchanan *et al.* (2005) in a larger population. Our hypothesis was that there would be an association between the genotypes and carcass traits.

3 MATERIALS & METHODS

3.1. Animal Samples

3.1.1. Behaviour Population

The Behaviour Population consisted of 400 crossbred steers purchased over the course of 5 days from an auction market near Saskatoon, Saskatchewan in 2005. The animals were initially housed at the University of Saskatchewan (U of S) beef research facility where they were treated identically and were fed the same backgrounding diet. They were then shipped to Pound-Maker Agventures where they were finished prior to being slaughtered at XL Beef in Moose Jaw, where Blue Tag data was collected.

A backgrounding diet (Pugh 2007) was fed to the steers for an average of 118 days depending on when they were purchased. At the time of processing individual weights were collected on each steer (SOB WT). The end of backgrounding (EOB) was March 31st, 2006. Live animal ultrasound was taken on April 5th and 6th to measure REA (EOB REA) and backfat (EOB BF) cover between the 12th and 13th ribs. The steers were weighed on May 2nd, which was recorded as the steer's start-of-finishing weight (SOF WT).

On May 3rd, the steers were shipped to Pound-Maker where they were housed in two pens of approximately 200 animals. Both pens were fed a standard finishing diet (Pugh 2007). End of finishing weights were July 31st and August 1, 2006 at 119 and 120 days on feed, respectively. The cattle were shipped to XL Beef in Moose Jaw in nine lots between September 5th and September 15th, where they were slaughtered and Blue Tag data was collected (Appendix A).

The following data were collected on the animals at either the backgrounding stage (U of S), finishing stage (Pound-Maker Agventures) or slaughter (XL Beef): Start-of-backgrounding weight (SOB WT), end-of-backgrounding weight (EOB WT), end-of-backgrounding back fat (EOB BF), end-of-backgrounding rib-eye area (EOB REA), start of finishing (SOF WT) and end of finishing (EOF). Backgrounding average daily gain (B-ADG) and finishing average daily gain (F ADG) were then calculated.

The Blue Tag data included hot carcass weight (HCW), average fat, grade fat, carcass rib-eye area (REA), marbling (Appendix B), and cutability. The average fat was calculated by taking the mean value of 3 fat measurements taken along the 12th rib *longissimus dorsi* muscle. The grade fat is a measurement taken from the narrowest fat depth over the fourth quadrant. Cutability is the yield estimate of the percentage of the carcass that is red meat.

3.1.2. Unrelated Animals

Allele frequencies were determined using 50 unrelated animals from the five major beef cattle breeds and one dairy cattle breed. The breeds included: Charolais, Angus, Simmental, Limousin, and Hereford and 20 cattle from the Holstein breed.

3.1.3. Tissue Samples

Samples were obtained from a 19-month old steer from an earlier study (Goodall and Schmutz 2007). The samples included kidney, testes, liver, rumen, muscle, adrenal, brain, small intestine, abdominal, lymph and thymus. Thymus samples from five calves that were 1 to 2 days old, which died unexpectedly, were collected within minutes of their death and immediately placed in RNA*later*® (Ambion, Austin, TX).

3.2. DNA and RNA Extraction

Genomic DNA from the Behaviour population and unrelated animals had been previously extracted from blood using the method described by Montgomery and Sise (1990). The isolation of total RNA and synthesis of the cDNA from the 19- month old steer tissues was also previously performed by Goodall and Schmutz (2007). Total RNA was isolated from the five-thymus samples following a protocol from Invitrogen (Burlington, ON.). Subsequently, cDNA was synthesized using the First Strand cDNA Synthesis Kit from Fermentas (Burlington, ON).

3.3. *POMC* Genotyping

3.3.1. Genomic

There were three methods used for genotyping *POMC*. For genotyping *POMC* c.288C>T both real time and PCR-RFLP were used, and for detecting whether or not the deletion existed, samples were separated on a 4% agarose gel.

3.3.1.1.Real time PCR of *POMC* c.288C>T

A LightCycler 1.0 (Roche Molecular Biochemicals) was used to perform the real time PCR. The following primers and probes were used:

Forward primer: 5'GATGAGCAGCCGCTGACT 3'

Reverse primer: 5'GTCAGCTCCCTCTTGAATTCGAG3'

Anchor: 5'GCTTCGGCCGTCGGAATGGT3'

Sensor: 5'GCAGCAGCAGCGGAGTTG3'

Total master mix volume was 10.3 µl, consisting of 5.6 µl of distilled water, 3 mM of $MgCl_2$, 4 pmol of forward and reverse primers, 3 pmol of both anchor and sensor probes and 0.7 µl of LightCycler DNA Master HybProbe (Roche Molecular Biochemicals). In each reaction 1µl of DNA sample template was used.

PCR conditions consisted of a denaturation step at 95°C for 10 min, followed by 45 cycles of 95°C for 2s, 60°C for 10s, and 72°C for 14s. The melting program consisted of heating the reaction to 95°C for 0s and then cooling to 40°C for a period of 120s, followed by a continuous increasing temperature transition rate of 0.2°C/s until 75°C is reached. The cooling segment of the program was lowering the temperature to 40°C for 5s.

The donor probe (anchor) was labeled with flourescein and the receptor probe (sensor) was labeled with LightCycler Red 640 (TIB Molbiol LLC, Adelphia, NJ). The sensor is the acceptor probe for the FRET reaction. Each genotyping run contained a maximum of 28 samples and three positive controls (TT, CT and CC) as well as a negative water control.

3.3.1.2. PCR-RFLP of *POMC* c.288C>T

Primers for the PCR-RFLP protocol were as described by Thue and Buchanan (2003). The PCR was carried out on a RoboCyler® (Stratagene® La Jolla, CA) followed by digestion with *BtsI* endonuclease (New England BioLabs Inc) and electrophoresis on a 2% agarose gel. The undigested PCR product (C allele) is 390 bp, while the digested T allele products are 233 bp and 157 bp in size.

3.3.1.3. Twelve bp deletion detection

New sets of primers were designed to test for the presence/absence of the deletion. The smaller product size from the new primers was necessary to ensure separation of PCR products on an agarose gel. The deletion PCR was performed on the LightCycler (Roche Molecular Biochemicals) using the following primers:

Forward (For3): - 5' CCG TCG GAA TGG TAG CA 3'

Reverse (Prev): - 5' CGT TGG GGT ACA CCT TC 3'

Total reaction volume was 10 µl, consisting of 3 mM of $MgCl_2$, 6 pmol of forward and reverse primers, 1.15 M Betaine and 0.7 µl of LightCycler DNA Master HybProbe (Roche Molecular Biochemicals). In each reaction 1µl of DNA sample template was used. PCR amplification program consisted of a denaturation at 95°C for 10 min, followed by 37 cycles of 95°C for 2s, 59°C for 10s, and 72°C for 14s. To cool the reaction the temperature was lowered to 40°C for 5s. PCR products were then separated on a 4% agarose gel. PCR products without the deletion are 206 bp in size and PCR products with the deletion are 194 bp in size.

3.3.2. cDNA

PCR was performed on cDNA from all of the tissues from the 19-month old steer using a RoboCycler® (Stratagene®). The forward primer resides in exon 1 while the reverse is in exon 3, this resulted in an amplicon that spanned introns 2 and 3 and would delineate cDNA from genomic DNA.

Forward (POMCEx1): 5'GCG ACG GAA GAG AAC GAA GGA 3'
Reverse (POMC3cDNARev2): 5'TGA TGG CGT TTT TGA ACA GCG T GAC 3'

The total reaction volume used was 15 µl, consisting of 5 pmol per reaction of forward and reverse primers, 200 µM dNTPs, 10X PCR Buffer (Fermentas), 1.5 mM $MgCl_2$, 1.3 M betaine, 6.1µl distilled water, 0.5 U *Taq*DNA polymerase (Invitrogen), and 1µl of cDNA template. The PCR started with denaturation at 94°C for 2 minutes, followed by 38 cycles of 94°C for 1 min; 53°C for 45s, and 72°C for 1 min, and finished with an extension of 72°C for 3 min. PCR products were then analyzed on a 2% agarose gel. Tissues showing expression of *POMC* were identified by visualization of an 858 bp product after electrophoresis.

3.4. Sequencing Panel with 20 cattle of specific genotypes

After the discovery of the deletion, 20 control animals were used to characterize the association between the deletion and *POMC* c.288C>T SNP. The animals consisted of 5 with the deletion, 5 TT, 5 CT and 5 CC at *POMC* c.288C>T. This panel of animals was sequenced using 5 different combinations of primers. Their genotype at the SNP and the presence/absence of the deletion was determined within each amplicon. Five sets of primers (Table 3.1) were necessary since it was initially thought that a pseudogene might also be present, containing both the T allele and the deletion. A primer map displaying the primer sets in relation to each other can be seen in Figure 3.1.

PCR products of these 20 cattle were cut out of the agarose gel and extracted using the protocol described in the QIA quick Gel extraction kit (QIAGEN, Mississauga, ON). Two microlitres of the extracted product and 1 ul of Ficoll Loading buffer were run on a 1.5% agarose gel along side a DNA mass ladder (Gibco) for quantification. The purified samples were then sent to the National Research Council of Canada Plant Biotechnology Institute, Saskatoon, SK for sequencing. Sequences were then analyzed using the Sequencher^{TM} program (Version 4.1. Gene Codes Corporation).

Table 3.1 Sequence of the primers used in the 20 animal panel for characterization of deletion

Primer Name	Primer Sequence	TM (^{O}C)
Thue and Buchanan Forward	CGT GCA TCC GGG CCT GCA AGC	72
Thue and Buchanan Reverse	GTCAGC TCC CTC TTG AAT TCG AG	70
Pseudo F	CGG AGG GAG TGG AAG GCT	60
Pseduo ER2	ACC CGT CTC GGC CGT CAT C	64
Pseudo R	GAG GCC TTC AGG GTC AAC	68
IntronBFwdMatch	TAT AGC TGC CGG GCA GGG GTA	58
POMC3cDNARev3	CCT TCT TGT GGG CGT TCT TGA TG	70

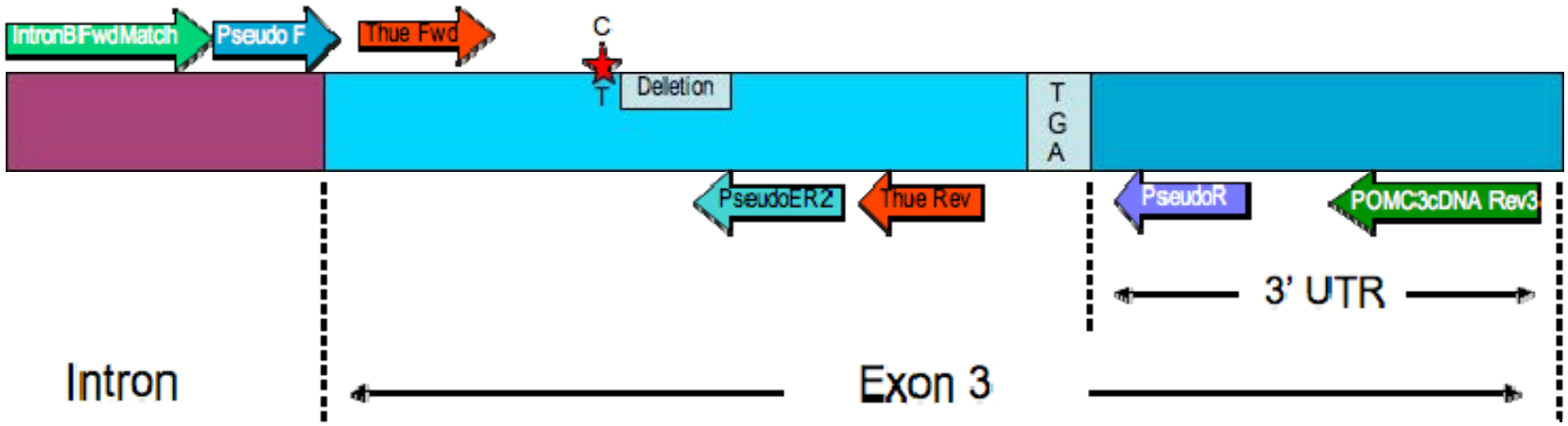

Figure 3.1 The positions of primers from Table 1 relative to each other. The ★ denotes *POMC* c.288C<T SNP

3.1. Statistical analysis

3.1.1. Deletion

A Mann Whitney U test was used to compare the production and carcass data of animals that had the deletion to those without the deletion. Only animals that were CT for the *POMC* c.288C>T SNP were analyzed. Deviations from the Hardy Weinberg Equilibrium were analyzed (Appendix C). A Chi square analysis using Statview 5.0 was also performed to test for significant differences between allele frequencies amongst the six breeds.

3.1.2. SNP

When analyzing for an association between the SNP and production and carcass traits, animals with the deletion were removed from the data set. Normality was determined using Shapiro-Wilk test scores. Parametric statistics were then performed using SAS 9.1 (2003). The association between genotype and carcass/production traits was analyzed using the mixed procedure to calculate a one-way analysis of variance (ANOVA) and the least-squared means (LSM). Using Proc Mixed, the finishing pen and kill dates were initially taken into account, but were removed subsequently from the model because there was no significant interaction.

To determine the effect of *POMC* genotype on various traits the following model was used:

$$Yij = \mu + POMCi + eij$$

where Y_{ij} was the dependent variable for the i_{th} observation, μ represents the mean of the dependent variable while $POMC_i$ is the effect of *POMC* genotype on the dependent variable, and e_{ij} represents the random error for each animal observed. Significance was defined at $P \leq 0.05$.

4 RESULTS

4.1. Discovery of a 12 bp deletion

The T allele has a melting temperature (Tm) of 61°C and the C allele a Tm of 66°C (Figure 4.1). A heterozygous animal that is CT is represented by the inclusion of both C and T allele peaks. During optimization of a real time PCR assay for *POMC* c.288C<T, some cattle exhibited an additional melting curve at 56°C (Figure 4.1). Samples exhibiting the additional melting curve were sequenced and a 12 bp deletion was discovered.

The deletion begins 5 bases downstream from *POMC* c.288C>T and results in the removal of 4 amino acids from the translated sequence. The 12 base pair deletion does not cause a frame shift, as one might expect with a deletion starting within a codon because it is divisible by three. The sequence instead comes back into frame. The G from the first position in the valine codon (residue 98) combines with the CC from the second and third positions of the second alanine codon (residue 102) to recreate an alanine that brings the sequence back into frame. The amino acids removed are valine, two glycines, and alanine, in this order (

Figure 4.2). We submitted sequence containing the 12 bp deletion to GenBank (accession number GQ280285; Appendix D).

In order to determine the presence/absence of the deletion, PCR-amplified products were electrophoresed in a 4% agarose gel (Figure 4.3). Lanes 2, 4 and 5 represent heterozygous animals with products at 206 bp and 194 bp (deletion).

4.2. *POMC* c.288C>T

Figure 4.4 depicts a representative gel of the PCR-RFLP used to genotype the animals for *POMC* c.288C>T. The C allele or undigested PCR product is 390 bp, the T allele is digested by *Bts*I into 233bp and 157bp fragments.

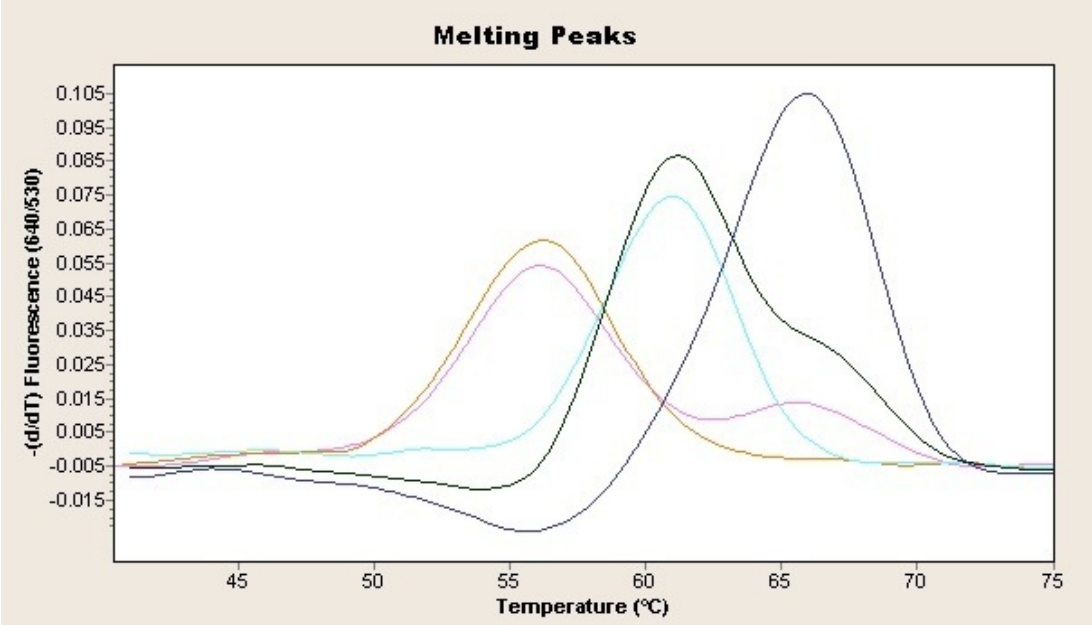

Figure 4.1 Melting temperature peaks from the LightCycler 2.0. Tm at 61°C denotes the T allele, Tm at 66°C denotes the C allele. Tm at 56°C represents an abnormal melting curve from the *POMC* c.288C>T genotyping assay

a)

AGC GGA GTT GGG GGC GCG GCC CAG AAG CGC GAG GAG

S	G	V	G	G	A	A	Q	K	R	E	E
96	97	98	99	100	101	102	103	104	105	106	107

b)

AGC GGA GCC CAG AAG CGC GAG GAG

S	G	A	Q	K	R	E	E
96	97	98	99	100	101	102	103

Figure 4.2 Partial sequence from exon three of *POMC*. a) highlights the twelve base pairs that are deleted and the affected amino acids in red, the first position in the codon coding valine and the 2nd and 3rd positions of the second alanine are coloured blue and the SNP is coloured green. Purple represents the KR cleavage site b) the same sequence minus the 12 bps and 4 amino acids showing how the DNA and protein sequence comes back into alignment

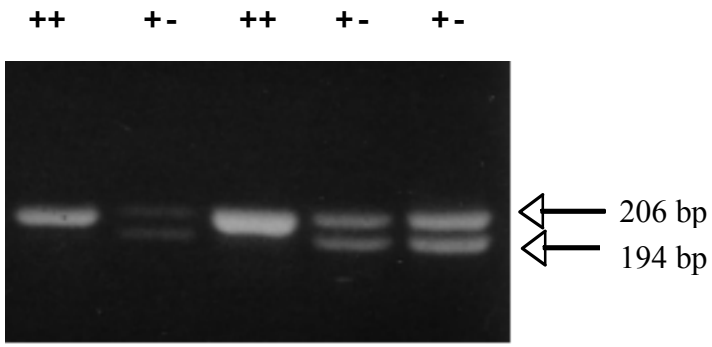

Figure 4.3 A representative gel showing the method used to detect the presence or absence of the deletion. The absence of the deletion (+) or the 12 bp deletion (-) in the *POMC* gene from crossbred steer genomic DNA. The 206 bp band represents *POMC* with no deletion and the 194 bp band represents *POMC* with the deletion

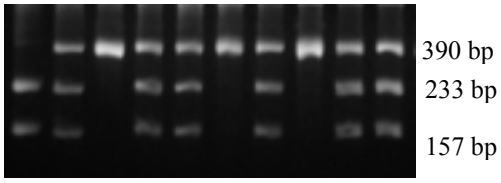

Figure 4.4 *POMC* PCR-RFLP gel showing genotypes. Lane 1 represents a TT animal, lanes 2, 4, 5, 7, 9 and 10 represent CT and lanes 3, 6 and 7 represent CC

4.3. Characterization of Deletion and SNP (Panel of 20 animals)

The SNP and deletion genotyping results from amplifying several overlapping segments of *POMC* in the panel of 20 animals are shown in Table 4.1. Genotyping results of the SNP and deletion using Thue and Buchanan primers are displayed in the second and third columns, respectively, of Table 4.1. Deletions are often found in either processed (typically lack introns) or non-processed pseudogenes. To characterize the deletion, a forward primer in intron 2 (PseudoF) was designed to determine if the observed deletion was part of a processed pseudogene. Using this intronic primer, if the deletion was lost it could be hypothesized that the deletion was in a processed pseudogene. This occurred in the first five cattle when using PseudoF and PseudoER2 primers and can be seen in the fourth and fifth columns. SNP genotypes that were previously recorded as being CT and containing the deletion with the Thue and Buchanan primers were now displaying a CC genotype with no deletion. The loss of the T allele and deletion supported the idea of a processed pseudogene that contained both the deletion and SNP. However, two TT animals remained TT (CCA153 and Gould 146) making the above theory incorrect.

Table 4.1 *POMC* SNP and deletion genotypes from different amplicons using 20 control animals

Animal [1] ID	Thue and Buchanan (2003) Fwd and Rev 390bp		PseudoF PseudoER2 381 bp		PseudoF PseudoR 858 bp		IntronBFwdMatch PseudoER2 486 bp		IntronBFwdMatch POMC3cDNARev3 1086 bp	
	[2] SNP	[3] Deletion*	[4] SNP	[5] Deletion**	[6] SNP	[7] Deletion**	[8] SNP	[9] Deletion**	[10] SNP	[11] Deletion**
CCA 2	C/T	+/-	C/C	+/+	C/C	+/+	C/T	+/-	C/T	+/-
1203	C/T	+/-	C/C	+/+	C/C	+/+	C/T	+/-	C/T	+/-
OR1947	C/T	+/-	C/C	+/+	C/C	+/+	C/T	+/-	C/T	+/-
OR1946	C/T	+/-	C/C	+/+	X	+/+	C/T	+/-	C/T	+/-
OR1942	C/T	+/-	C/C	+/+	C/C	+/+	C/T	+/-	C/T	+/-
CCA 103	T/T	+/+	X	+/+	X	X	T/T	+/+	T/T	+/+
CCA 153	T/T	+/+	T/T	+/+	X	X	T/T	+/+	T/T	+/+
Gould 48	T/T	+/+	X	+/+	X	X	T/T	+/+	T/T	+/+
Gould146	T/T	+/+	T/T	+/+	X	X	T/T	+/+	T/T	+/+
Gould145	T/T	+/+	X	+/+	X	X	T/T	+/+	T/T	+/+
CCA 7	C/T	+/+	C/C	+/+	C/C	+/+	C/T	+/+	C/T	+/+
CCA 8	C/T	+/+	X	+/+	C/C	+/+	C/T	+/+	C/T	+/+
CCA 9	C/T	+/+	C/C	+/+	C/C	+/+	C/T	+/+	C/T	+/+
CCA 10	C/T	+/+	C/C	+/+	C/C	+/+	C/T	+/+	C/T	+/+
CCA 11	C/T	+/+	C/C	+/+	C/C	+/+	C/T	+/+	C/T	+/+
CCA 152	CC	+/+	C/C	+/+	C/C	+/+	C/C	+/+	CC	+/+
Gould 215	CC	+/+	C/C	+/+	C/C	+/+	C/C	+/+	CC	+/+
Gould 258	CC	+/+	C/C	+/+	C/C	+/+	C/C	+/+	CC	+/+
Gould 261	CC	+/+	C/C	+/+	C/C	+/+	C/C	+/+	CC	+/+
Gould 252	CC	+/+	C/C	+/+	C/C	+/+	C/C	+/+	CC	+/+

+/+	No deletion present
+/-	heterozygous deletion
*	Detection of deletion by running product out on gel and seeing 12 bp separation
**	Detection of deletion by DNA sequencing
X	Unusable sequence
Note	Superscript numbers denote column designation

An additional assay was designed to characterize the changing genotypes. A reverse primer (PseudoR) located further downstream in the 3' untranslated region was designed (Table 3.1). Non-processed pseudogenes are typically truncated, and hence by utilizing a reverse primer farther downstream it was possible that the primer would be outside of the duplicated region and no longer amplify the pseudogene. However, using PseudoF with the PseudoR did not yield CT or deletion genotypes in any of the cattle, as shown in columns six and seven (Table 4.1).

To verify which allele was being expressed, calves that were CT in their genomic DNA were genotyped using thymus cDNA. Their genotype remained CT, verifying that the SNP was being expressed in the real gene.

A BLAST search of the target region of *POMC* with traces in GenBank, resulted in numerous unannotated sequences being found, one of them being a sequence named F Trace. F Trace appears to have similar homology to the reference *POMC* sequence (AY940127) that was used to design primers. There are some differences between the two sequences (Figure 4.5). The forward primer (PseudoF) matches up with the reference sequence but it also likely amplified F Trace as only three bases at the start of the primer differ. When using the PseudoF primer it was usually difficult to obtain good amplification and sequence.

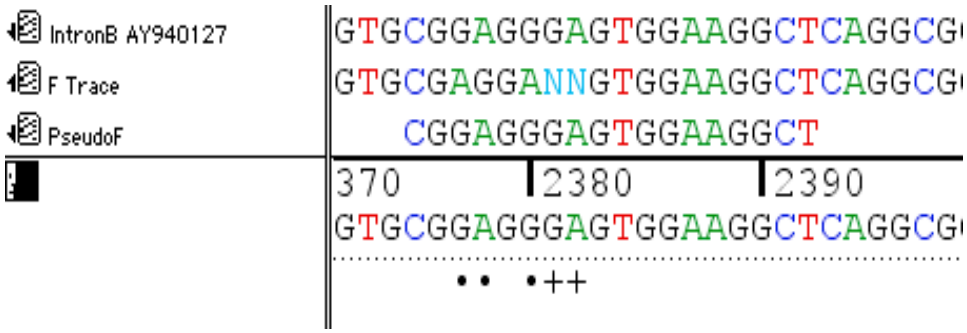

Figure 4.5 Sequence showing the similarities between PseudoF primer and both AY940127 and F Trace. A (.) represents the positions where sequence between PseudoF and F Trace are different

The similarity of F Trace with our reference sequence (IntronB AY940127) may have resulted in the amplification of both *POMC* and F Trace. Therefore, what was thought to be a pseudogene was probably F Trace. Thus the CT animals were changing genotypes at *POMC* c.288C>T SNP because there were two products being amplified. At the position of *POMC* c.288C>T in F Trace the sequence has a C allele.

A new forward primer was designed, IntronBFwdMatch, which has 100% homology with the *POMC* intron 2 reference sequence (AY940127). This primer was designed to have five mismatch bases with F Trace, three of which are at the end of the primer creating a 3' loose end. Having the 3' loose end ensured only the desired target was amplified (Figure 4.6).

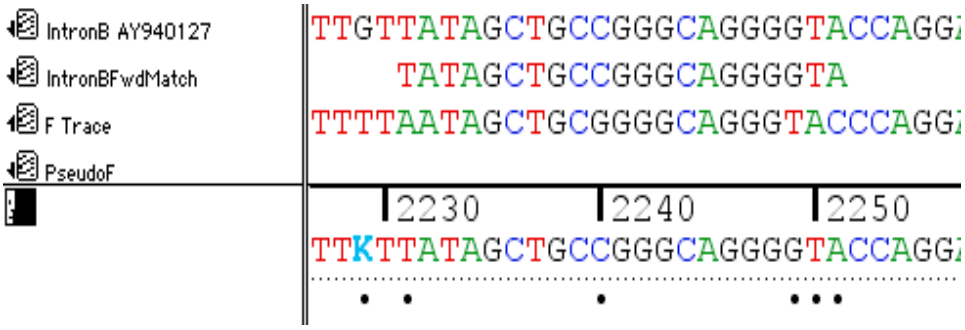

Figure 4.6 Sequence showing new primer that has a 100% homology with IntronB reference strand and 5 base pair differences with F Trace

The results of IntronBFwdMatch and PseudoER2 primers are shown in columns eight and nine (Table 4.1). There were no genotype changes within the *POMC* c.288C>T SNP or the deletion when using these primers as compared to when we used the Thue and Buchanan primers. To confirm these results and rule out a non-processed pseudogene another reverse primer was designed, POMC3cDNARev3, to pair with the forward primer IntronBFwdMatch. Column ten and eleven report these results, which are the same as column 8 and 9 with respect to the SNP and deletion (Table 4.1).

The poor amplification we experienced with some of the animals (column 4 and 6 in Table 4.1) was due to a SNP within the PseudoF primer. This SNP was identified in the *POMC* sequence used to design the PseudoF primer. The SNP is a G to T base substitution (g.2424T>G). In the PseudoF primer there was a T in the position of the SNP. Within the panel

of 20 animals, the T allele was always found with the C allele at the c.288C>T SNP and hence why we observed good amplification of CC and CT animals. Whereas the G allele at this SNP was always found with the T allele at c.288C>T and hence why the homozygous T animals were difficult to amplify (

Table 4.2).

Use of the IntronBFwdMatch primer generated "good" amplification and sequence indicating that F Trace was not being amplified. These results were verified through sequence alignment in Sequencher™.

Table 4.2 Genotype comparison of *POMC* c.288C>T SNP and SNP within PseudoF primer (g.2424T>G). The table shows the presence of the T allele of the *POMC* SNP with the G allele

Animal ID	c.288C>T SNP	g.2424T>G
CCA 103	T/T	G/G
CCA 7	C/T	G/T
CCA 152	C/C	T/T

4.3.1. mRNA Study

A screening of mRNA's from different tissues was performed to determine if *POMC* expression was present. *POMC* was only present in the thymus, of the 11 tissues from the 19-month old steer that were studied (Figure 4.7).

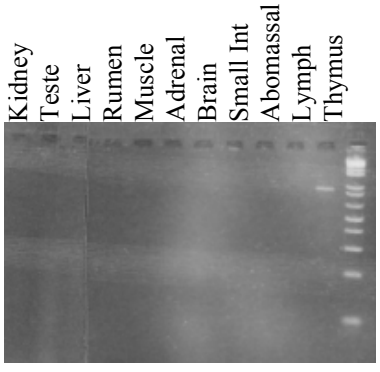

Figure 4.7 *POMC* PCR amplification using cDNA isolated from various tissues

4.4. Deletion

4.4.1. Allele Frequency

There were 14 animals identified with the deletion (Table 4.3). The allele frequency for no deletion was 0.98 and with the deletion was 0.02. The individual deletion genotypes from the Behaviour animals are presented in Appendix A.

Table 4.3 Deletion genotype frequencies and occurrence in 386 Behaviour animals

Genotype	**+/+**	**+/-**	**-/-**
n =	372	14	0
Frequency	0.96	0.04	0

4.4.3 Hardy Weinberg Equilibrium

Due to the absence of homozygous deletion animals and the low deletion allele frequency, it was important to test if homozygotes were expected based on the Hardy Weinberg Equilibrium expected values. The observed and expected values did not differ (Appendix C).

4.4.2. Allele Frequency in five breeds

The frequency of the deletion varied among Angus, Hereford, Simmental, Charolais, Limousin and Holsteins (

Table 4.4). A Chi squared analysis revealed there was no significant difference in allele frequency among breeds (P>0.05).

4.4.3. Association Study

Mann-Whitney U tests were performed for traits that compared animals heterozygous for the deletion to homozygous non deletion animals. All of these animals were CT for the *POMC* c.288C>T SNP. Results are presented in Table 4.5. Animals homozygous for not having the deletion showed a significant increase in carcass REA and a trend in EOB-REA. The Carcass REA of animals without the deletion was 7.91 cm^2, larger than the animals with the deletion.

Table 4.4 Deletion genotypes and allele frequency of the deletion in six major breeds

Breed	n	+/+	+/-	-/-	Minor Allele Frequency of Deletion
Angus	50	46	4	0	0.04
Hereford	50	48	2	0	0.02
Simmental	50	48	2	0	0.02
Charolais	50	49	1	0	0.01
Limousin	50	48	2	0	0.02
Holstein	20	18	2	0	0.05

(+) denotes no deletion present and (-) denotes occurrence of deletion

Table 4.5 Association analysis of the carcass traits in the 151 CT crossbred steers with *POMC* c.293_304delTTGGGGGCGCGG using a Mann-Whitney U (LSM)

Trait	+/+ (n = 138)	+/- (n = 13)	P- value
SOB WT (kg)	246.3	240.4	0.23
EOB WT (kg)	389.7	385.1	0.50
B-ADG	1.21	1.20	0.81
EOB BF (mm)	2.29	2.26	0.88
EOB-REA (sq cm^2)	65.20	60.92	*0.08*
SOF WT (kg)	435.5	424.7	0.28
EOF WT (kg)	639.6	626.1	0.42
F ADG	2.27	2.94	0.55
HCW (kg)	382.5	369.5	0.15
Average Fat (mm)	9.39	10.08	0.38
Grade Fat (mm)	7.86	8.61	0.28
Carcass REA (cm^2)	103.6	95.69	**0.02**
Marbling	7.79	7.54	0.13
Cutability	61.38	60.62	0.47

SOB=Start of backgrounding, WT=Weight, EOB=End of backgrounding, EOF= End of Finishing, ADG=Average Daily Gain, BF=Backfat, REA=Rib-eye Area, HCW=Hot Carcass Weight, F=Finishing Significance was P<0.05 and a trend P<0.1

4.5.

4.5. *POMC* c.288C>T

4.5.1. Allele frequency

The allele frequencies were 0.75 and 0.25 for the C and T allele respectively. Genotype frequencies are reported in Table 4.6.

Table 4.6 Genotype frequencies of SNP in the Behaviour animals

Genotype	CC	CT	TT
n =	210	138	24
Genotype Frequency	0.56	0.37	0.06

4.5.2. Association Study

Based on Shapiro-Wilk test scores for some traits the data were not normally distributed. These traits included EOB-REA, average fat, grade fat, marbling, cutability, and EOB BF, all of which had a $P \leq 0.05$ for normality.

Therefore the non-parametric statistic, Kruskal-Wallis test, using Proc NPAR1WAY was performed based on the normality results. Parametric statistics were run to obtain LS Means. This analysis is appropriate if there is a large sample size (Williams and Nernez 1995). Proc Mixed was used to see if *POMC* genotype had an association with production or carcass trait. Significance stayed the same for all traits except cutability, which changed from a trend to significance. The results of the one-way ANOVA are presented in Table 4.7.

While both SOF WT and HCW were overall significant, significance was only observed between the CC's and CT's. Average fat and grade fat both numerically decreased with each addition of a T allele. With average fat only the CC's and TT's were significantly different from each other, with grade fat only the CC's and CT's were significantly different from each other. Carcass REA numerically increased also with the addition of a T allele, however only the CC's and CT's were statistically different from each other. Marbling significantly increased with the addition of one T allele. Cutablility showed a trend with a P-value of 0.06. Individual genotype results can be seen in Appendix A.

Table 4.7 Association analysis of carcass traits for 372 crossbred steers with *POMC* c.288C>T SNP (LSM(±SEM))

Trait	CC n= 210	CT n=138	TT n=24	Parametric P-Value	Non-Parametric P-Value
SOB WT (kg)	243.04 ± 1.34	246.31 ± 1.66	242.21 ± 3.97	0.27	0.43
EOB WT (kg)	383 .74 ± 2.06	389.75 ± 2.54	384.08 ± 6.10	0.18	0.26
B-ADG	1.19 ± 0.01	1.21 ± 0.02	1.20 ± 0.04	0.56	0.58
EOB BF (mm) [1]	2.31 ± 0.10	2.19 ± 0.12	2.24 ± 0.28	0.74	0.61
EOB-REA (cm^2) [1]	64.09 ± 0.52	65.20 ± 0.64	67.02 ± 1.53	0.12	0.11
SOF WT (kg)	426.61 ± 2.24 [b]	435.54 ± 2.76 [a]	427.63 ± 6.62 [ab]	**0.04**	**0.05**
EOF WT (kg)	628.11 ± 3.75	639.58 ± 4.60	624.26 ± 10.98	0.12	0.15
F ADG	2.27 ± 0.02	2.30 ± 0.03	2.22 ± 0.07	0.45	0.55
HCW (kg)	373.64 ± 1.98 [b]	382.53± 2.44 [a]	376.93 ± 5.86 [ab]	**0.02**	**0.02**
Average Fat (mm) [1]	10.09 ± 0.25 [a]	9.39 ± 0.31 [ab]	8.46 ± 0.74 [b]	**0.05**	**0.03**
Grade Fat (mm)[1]	8.67 ± 0.25 [a]	7.86 ± 0.31 [b]	7.33 ± 0.74 [ab]	**0.05**	**0.04**
Carcass REA (cm^2)	100.40 ± 0.81 [b]	103.63± 0.10 [a]	104.04 ± 2.40 [ab]	**0.03**	**0.01**
Marbling [1]	7.65 ± 0.04 [b]	7.79 ± 0.05 [a]	7.91 ± 0.12 [a]	**0.02**	**0.03**
Cutability (%)	60.83 ± 0.21	61.38 ± 0.27	62.18 ± 0.65	*0.06*	**0.02**

[1] These traits were not normally distributed.

[ab] Means in the same row with different superscripts are significantly different (P≤0.05)

SOB WT=start of Backgrounding, WT-Weight, EOB WT=End of Backgrounding, B=Backgrounding, ADG=Average Daily Gain BF=Backfat, REA=Rib-eye Area, SOF=Start of Finishing, EOF=End of finishing, F=Finishing, HCW=Hot Carcass Weight

5 DISCUSSION

5.1. Deletion

A 12 bp deletion in exon 3 of bovine *POMC* was identified (

Figure 4.2), which results in the removal of 4 amino acids. Sequence alignment using genomic DNA confirmed that the deletion occurs in the γ-MSH peptide of cattle (Figure 5.1). This figure shows that the γ-MSH sequence is relatively conserved in the 5' end of mammals, however, not so much in the 3'. This is why the short forms are still active. Tung *et al.* (2006) demonstrated that γ^3-MSH was more effective than γ^2-MSH at reducing food intake.

A similar 12 bp deletion in the human c-kit protooncogene, was discovered by Riva *et al.* (1995). Like the *POMC* deletion, c-kit protooncogene deletion removes 4 amino acids. The deletions were similar in that both deletions started in the second nucleotide of the codon and ended with the first nucleotide of the 4th amino acid resulting in the recreation of an alanine. Rival *et al.* (1995) did not determine the implication of this deletion.

In 14 animals the *POMC* deletion occurred in conjunction with the T allele at the *POMC* c.288C>T SNP. In 162 animals the T allele was identified without the deletion. This would support the theory that the deletion evolved after the SNP.

5.2. Expression

The expression study using mRNA extracted from 12 tissues from a 19-month-old steer determined that *POMC* was synthesized in the thymus (Figure 4.7). Using this knowledge we obtained thymus samples from recently deceased calves, which were used to determine the allele that was being expressed at the *POMC* c.288C>T SNP. There was no housekeeping gene used and therefore we can not conclude that these tissues do not ever express *POMC.* Chen *et al.* (2000) found expression of a pro-opiomelanocortin-like gene in the testis and epididymis of rat and mouse, as well as in the pituitary and brain. We did not observe any expression in testis in our cattle samples. Contrary to our findings, Pintar et al. (1984) also found *POMC* expressed in the ovaries and testis of rat as well as the adrenal medulla and spleen. Kono *et al.* (2001) also identified *POMC* mRNAs in the basal layer of human skin.

A. γ-MSH peptides

γ^3-MSH:	YVMGHFRWDRFGPRNSSSAGGSAQ
γ^2-MSH	YVMGHFRWDRFG
γ^1-MSH	YVMGHFRWDRF-NH_2

B. Alignment of γ-MSH peptides of various species

MAMMALS

CATTLE	YVMGHFRWDRFGRRNGSSSSGVGGAAQ
CATTLE DELYVMGHFRWDRFGRRNGSSSSG	.....AQ
SHEEP	YVMGHFRWDRFGRRNGSSSFGAGGAAQ
PIG	YVMGHFRWDRFGRRNGSSSGGGGGGGAGQ
RAT	YVMGHFRWDRFGPRNSSSAGGSAQ
MOUSE	YVMGHFRWDRFGPRNSSSAGSAAQ
DOG	YVMGHFRWDRFGRRNSSSSGSAGQ
HUMAN	YVMGHFRWDRFGRNSSSSGSSGAGQ

Figure 5.1 Peptide sequence of rat γ-MSHs and alignment with other mammalian γ-MSH **(A)** Rat γ-MSH sequences. **(B)** Alignment of γ-MSH peptides of mammals. Red color indicates position of *POMC* c.288C>T SNP. Highlighting indicates variations from cattle sequence (modified from Denef *et al.* 2003)

5.3. Allele Frequencies

The allele frequencies for *POMC* c.288C>T are 0.25 and 0.75 for the T and C allele, respectively, in our population of 372 crossbred steers. These results are consistent with the finding of Buchanan *et al.* (2005). They observed allele frequencies of 0.23 and 0.77 for the T and C allele, respectively, in 256 Charolais-cross steers. The allele frequencies reported here verify the original frequencies found are prevalent in other breeds and are not limited to Charolais-cross animals.

The allele frequency for the presence or absence of the 12 bp deletion was determined in five beef breeds common in Canada and one dairy breed. These included Angus, Hereford, Simmental, Charolais, Limousin and Holstein (

Table 4.4).

The deletion frequency was highest in the Holstein breed at 0.05. Angus was the next highest at 0.04, followed by Hereford, Simmental, and Limousin which all had a frequency of 0.02. The lowest frequency was found in Charolais at 0.01. Although the allele frequencies vary among these breeds, a Chi squared analysis revealed that there is no significant difference between breeds.

5.4. Characterization of the Deletion

In the early stages of the deletion discovery it was postulated that one or both the deletion and the T allele from the (*POMC* c.288C>T) SNP might be located within a processed pseudogene, which do not have introns (D'Errico *et al.* 2004). Therefore, we designed an intronic primer to amplify the region containing the deletion and SNP. If the deletion and T

allele were lost then they were indeed within a processed pseudogene. With amplification of the product using the forward intronic primer (PseudoF) with PseudoER2 reverse, the deletion disappeared but the T allele remained in two animals (Table 4.1). This made it inconclusive as to whether or not the SNP was in a processed pseudogene.

The other hypothesis was that the deletion and/or SNP existed within a non-processed pseudogene. To move out of the potentially duplicated region, the reverse primer PseudoR was designed further downstream. Sequenced PseudoF and PseudoR PCR products again revealed that CT animals were changing to CC. PCR product from the TT animals could not be amplified

(Table 4.1). It was noted that when using either of these primer sets, the TT animals were always difficult to amplify. This was apparent on agarose gels where TT animals had lower intensity bands compared to CT and CC animals, indicating lower PCR product yield (Figure 5.2).

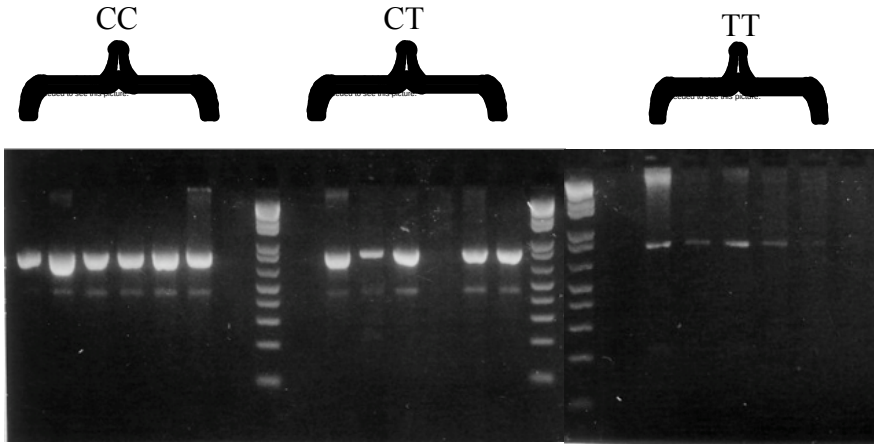

Figure 5.2 PCR product using PseudoF forward primer and PseudoER2 reverse primers. The intensity of the bands depicts the difference in product amounts between genotypes

While characterizing the deletion, a BLAST search of our *POMC* region revealed F Trace, an unannotated sequence. Performing an alignment in Sequencher™ revealed that the PseudoF primer (the intronic primer) was likely amplifying both the real *POMC* gene and F Trace. To eliminate the amplification of two products**,** a forward primer, IntronBFwdMatch, was designed to have 100% identity with the real gene and 5 purposeful mismatch bases with F Trace. This resulted in good amplification, and a clear readable sequence. With these primers, the genotypes of the SNP and deletion were the same as originally determined (Table 4.1). In order to explain why the T allele was not amplifying well, we re-analyzed the region under the PseudoF primer and found the g.2424T>G SNP. This SNP is located in intron 2 and is not near a donor or splice site and therefore would not have an effect on the gene processing.

The *POMC* c.288C>T SNP was identified by Thue and Buchanan in 2003, however they did not identify the deletion at this time. We would not have expected them to have seen the deletion on their agarose gels as at least a 4% agarose gel is need to resolve the 12 bp PCR product out of the larger PCR product.

5.5. Trace F Characterization

We speculate that if Trace F is a pseudogene, it occurred very recently. We did not find any difference between Trace F sequence and sequenced cDNA within exon 3. Point mutations were only found between the two sequences in intron 2. Further research would need to be completed in order to further characterize it as a pseudogene.

5.6. Association Study

5.6.1. Deletion

Using the Mann Whitney U test an association study was performed to determine the deletion's effect on carcass traits. Cattle heterozygous for the deletion had a significantly lower EOB-REA and carcass REA (Table 4.5).

Within the *POMC* peptide, the deletion is located in the γ-MSH peptide (Figure 5.1). It can be hypothesized that the location of the deletion and severity of losing four amino acids changes the structure of the peptide. These folding and structural changes could then be responsible for interfering with the gene processing of *POMC* products.

A 9 bp insertion exists in *POMC* in humans (Feng *et al.* 2003), and is in the vicinity of our 12 bp deletion in cattle, however it is not in γ-MSH. The 9 bp deletion in humans is located in the carboxyl terminus of *POMC*, the region from which γ-MSH is derived. Feng *et al.* (2003) did not find any significant associations between the 9 bp insertion/duplication with body composition in children. Feng *et al.* (2003) speculate that an in frame insertion located in an area of *POMC* that does not yield a peptide would not affect the production of ACTH or α-MSH. We speculated that because our deletion is within the γ–MSH coding region it may have effects on the production of ACTH or α-MSH, and therefore we would potentially observe an effect on the appetite pathway and ultimately carcass traits.

Sequence from a deletion animal was entered into a program called Predict Protein2008. The program revealed the loss of a glycosaminoglycan (GAG) site. GAG is predicted to bind to the amino acid sequence SGVG, but in the deletion animal the amino acid sequence becomes SGAQ instead. The loss of the GAG binding site could be important to the processing of POMC since GAG's have been shown to be involved in the secretory pathway through the Golgi apparatus (Fernandez and Warren, 1998). This pathway is where some post-translational modifications occur, such as prohormone convertases cleavage, and compartmentalization for

transport to the cell membrane (reviewed in Pritchard *et al.* 2002). Without the ability to bind GAG, *POMC* might not be able to properly pass through the Golgi to be cleaved by prohormone convertases or the prohormone might not be transported to the cell membrane for secretion. The deletion also occurs very close to one of the KR cleavage sites (Figure 4.1). Due to the removal of four amino acids there may be changes in the protein folding that could obscure prohormone cleavage, and result in some peptides not being generated.

We speculate that the removal of 4 amino acids and the changes to the binding site would alter the gene structure enough to interfere with the processing of the *POMC* and may decrease the viability of animals homozygous for the deletion. Yaswen *et al.* (1999) supported this theory because when they crossed heterozygous mice, only a quarter of the expected homozygous (lacking all *POMC*-derived peptides) were born. Implying the lack of *POMC* peptides is detrimental to survival. Krude *et al.* (1998) identified 2 mutations in humans that impede the processing of α-MSH and ACTH. This interference with ACTH resulted in adrenal insufficiency that was lethal without supplemented hydrocortisone administration (Krude *et al*. 1998). We can speculate that if the deletion interferes with the production of ACTH as well, left untreated the homozygous deletion cattle would not survive. Due to the low allele frequency of the animals we did not expect to observe any homozygous deletion animals. We can therefore not conclude that homozygous deletion animals would not survive. In practical terms if the deletion does result in low viability it would be important for breeders to select for cattle which do not posses the homozygous deletion.

It is presumed that γ-MSH is the ligand for MC3R (Roselli-Rehfuss *et al.* 1993). Chen *et al.* (2000), found that $MC3R^{-/-}$ mice had increased fat mass and higher feed efficiency. Since our deletion is likely to affect the production of γ-MSH it is surprising that our results did not support Chen *et al.* (2000) findings. We did not find a significant correlation with the deletion and either grade fat or average fat (

Table 4.4).

Our results demonstrate there may be an economical benefit for producers to identify animals that possess the deletion. In the case of purebred or cow/calf producers they could make breeding decisions that would potentially optimize the trait of REA growth potential. Depending on the target market, producers may choose to select cattle with a larger REA if they were paid premiums. One example of where producers would select for a smaller REA is if they were trying to achieve Certified Angus Beef (CAB) status. Parameters to achieve this grade include having a REA that is between 64.4 cm^2-103.22 cm^2 (Certified Angus Beef, 2009). Therefore, knowing you are likely to end up with a 7.91 cm^2 smaller REA, the deletion status is important. In our population 162 animals have a REA larger than 103 cm^2. If a producer was targeting these 162 animals to be CAB they would no longer meet the requirements and would loose considerable economic value.

5.6.2. *POMC* c.288C>T SNP

Of the significant carcass traits analyzed HCW is one of particular importance. The *POMC* c.288C>T SNP had an overall significant association with HCW (P=0.02). The T allele was associated with a significant 8.89 kg increase in weight between the CC and CT genotypes. The TT animals did not demonstrate the additive effect of the T allele nor did its mean value follow a numerical trend. These significant associations between the *POMC* c.288C>T SNP and HCW are similar to previous findings by Buchanan *et al.* (2005) who also found a significant difference between the CC and CT animals having a 7.4 kg increase in HCW but no difference between CT and TT. The associations with the *POMC* c.288C>T SNP and increase HCW coincides with previous research demonstrating mutations in *POMC* leads to early onset obesity and increased body weight (Echwald *et al.* 1999; Tung *et al.* 2006). Although the HCW association does not have an additive effect in our study the fact that there was significance found makes it worthy of future research. This is due to the importance of HCW in value determination for the carcass.

Both average fat and grade fat numerically decreased with the addition of each T allele, indicating that there is potentially an additive effect of the T allele. With average fat only the TT's and CC's were statistically different from each other. With grade fat the CC and TT animals were significantly different. Buchanan *et al.* (2005) study did not find any correlations

between the *POMC* c.288C>T SNP with either fat measurement. Previous research by Baker *et al.* (2005) identified genetic variations in *POMC* that influenced body fat distribution, which led them to suggest *POMC* plays a role in metabolic regulation. May *et al.* (1992) reported that back fat thickness is one of two traits most significantly impacting boneless subprimal yield and trimmable fat in beef carcasses. Given the effect of *POMC* genotype on back fat (average and grade fat) it is likely that *POMC* genotype will impact the Yield Grade and carcass value. This knowledge may be useful to both the packer industry and primary producer in determining animal value when purchasing/selling the animal.

Carcass REA is an important trait as it is considered when carcasses are being graded. We again had an additive effect with the addition of the T allele, but only the CC and CT were statistically different (Table 4.7). The SEM was higher in the TT animals, which potentially explains the lack of statistical significance. A larger sample population that would result in more TT animals would help to reduce this variation.

We found the T allele was associated with deceased marbling in an additive fashion (Table 4.7). However, this would not be enough to move a carcass into a lower marbling class. Forbes *et al.* (2001) showed additional support of the role that MSH and leptin have with metabolism and appetite in relation to fat stores.

With respect to the *POMC* c.288C>T SNP, further examination using a larger sample size would be beneficial. Having a large population to test would allow the researchers to ensure they obtained a higher number of the TT genotypes to analyze. Although the *POMC* c.288C>T SNP is a silent mutation, the fact that we found significance in several important carcass traits makes it worthy of future research. Resent research has demonstrated the due to several factors silent mutations are becoming more important. Kimchi-Sarfat *et al.* (2006) reported that a silent SNP in the Multidrug Resistance gene altered the inhibitor and drug interactions with the gene product P-glycoprotein. They hypothesized that this mutation although silent affected the protein folding and function. Silent mutations could also interfere with exonic splicing, because in changing the codon they may be altering the sequences cues for intron removal. Research has shown that there are more than 50 genetic disorders that are linked to silent mutations (Chamary and Hurst 2009). Another study by (Kudla, *et al.* 2006) demonstrated that G and C bases may increase mRNA synthesis. Since the *POMC* c.288C>T is a C to T substitution this may result in a reduced amount of POMC expression. As a consequence of this there would be an increased

appetite due to less α-MSH expression. Our findings support this theory, as we found an increase in HCW with the addition of one T allele (Table 4.7).

Future research could involve evaluating the effects of the deletion using a larger population. Using a sample population greater than 2500 animals would allow for the possibility of having some homozygous deletion animals. To further evaluate the viability of homozygous deletion animals a research study mating heterozygous deletion animals could allow for testing of weather homozygous deletion animals are viable or if their survival rate is less than a heterozygous animal. To determine if the deletion interrupts the processing of the POMC peptide or more specifically α-MSH expression a radioimmunoassay (RIA) could be performed. Lastly a study could also be performed which looked at the direct function of GAG in *POMC* processing and secretion.

6 CONCLUSION

A new significant correlation with the *POMC* c.288C>T SNP was identified in carcass-REA, grade and average fat, and marbling. Due to the low sample number of TT animals it would be beneficial to validate on a larger population. This research study also led to a new discovery. A 12 bp deletion was found in *POMC* within exon 3. Further analysis of the deletion showed a significant correlation with carcass traits, specifically resulting in smaller carcass EOB-REA and carcass REA. Since REA is a contributor to the economic value of a carcass it would be beneficial for producers to identify this variant in their herds. The practical utility of this knowledge may be in identification of animals with a genetic potential for smaller REA. This knowledge may lead to altering days to slaughter of these animals in order to optimize the fat:muscle ratio of the REA in order to maximize the yield grade score. Commercialization is likely to require a more robust evaluation of both the *POMC* c.288C>T SNP and the deletion.

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APPENDICES

Appendix A Genotypes & Blue Tag Data

Lab #	*POMC*	Deletion	HCW (kg)	Average fat	Grade fat	REA (cm^2)	Marbling	Cutability
06-001	CT	-/+	377.6	10	8	97	8	64
06-002	CC	-/-	392.6	4	4	115	8	64
06-003	TT	-/-	356.8	5	5	117	9	65
06-004	CT	-/-	425.0	8	7	112	8	64
06-005	CC	-/-	360.4	7	5	99	8	64
06-006	CT	-/-	350.4	9	7	86	8	62
06-007	CT	-/-	341.3	12	10	91	8	58
06-008	CC	-/-	460.6	13	11	108	7	58
06-010	CT	-/-	396.2	8	5	86	7	64
06-011	CC	-/-	389.9	14	10	114	8	61
06-012	CT	-/-	414.8	14	13	96	8	57
06-013	CT	-/-	370.3	7	5	117	8	64
06-014	CT	-/-	360.2	9	7	99	7	63
06-015	CT	-/-	400.5	6	5	116	8	64
06-016	CT	-/-	407.6	10	8	96	7	61
06-017	CT	-/-	380.0	3	2	116	9	65
06-018	CC	-/-	369.2	14	13	94	7	57
06-019	CT	-/-	413.5	10	9	108	8	61
06-020	CC	-/-	415.9	12	11	103	7	60
06-022	CC	-/-	383.5	7	6	111	7	62
06-023	CC	-/-	294.8	9	8	99	8	62
06-024	CC	-/-	367.1	12	10	104	8	60
06-025	CC	-/-	423.4	16	14	93	7	55
06-026	CT	-/-	386.9	8	7	97	7	62
06-027	TT	-/-	420.7	11	9	108	7	61
06-028	CC	-/-	381.7	16	15	98	7	58
06-029	CT	-/-	399.4	8	6	106	7	62
06-030	CT	-/-	431.6	14	12	95	7	57
06-031	CT	-/-	378.3	6	4	103	8	64
06-032	CT	-/+	374.2	7	5	104	8	64
06-033	CT	-/-	371.0	5	5	103	8	62
06-034	CT	-/-	372.9	6	5	99	7	64

Lab #	*POMC*	Deletion	HCW (kg)	Average fat	Grade fat	REA (cm^2)	Marbling	Cutability
06-035	CC	-/-	359.2	14	12	98	8	59
06-036	CT	-/-	332.0	3	2	98	8	65
06-037	CC	-/-	395.5	8	7	101	7	62
06-040	CT	-/-	379.0	16	11	98	7	59
06-041	CT	-/-	370.1	9	8	96	7	62
06-042	CC	-/-	392.4	8	9	93	7	59
06-043	CT	-/+	365.8	5	4	99	8	64
06-044	CC	-/-	371.0	18	20	85	7	51
06-045	TT	-/-	396.7	7	7	97	.	C
06-046	TT	-/-	342.0	5	4	109	8	65
06-047	CT	-/-	424.1	6	6	98	7	64
06-048	CT	-/-	444.1	7	7	104	8	62
06-049	CT	-/-	420.9	10	9	99	7	61
06-050	CT	-/+	406.4	8	7	109	8	64
06-051	CC	-/-	357.9	5	4	98	8	63
06-052	CC	-/-	347.0	8	6	103	8	64
06-053	CT	-/-	350.6	6	5	116	8	64
06-054	CC	-/-	320.9	15	14	80	7	57
06-055	CT	-/-	325.5	9	5	88	8	62
06-056	CC	-/-	376.0	10	7	102	7	62
06-057	CC	-/-	427.3	11	9	110	8	61
06-058	CT	-/-	389.4	19	20	76	7	50
06-059	CT	-/-	350.9	3	3	98	9	64
06-060	CC	-/-	396.7	14	14	95	8	55
06-061	CC	-/-	442.0	9	7	124	8	62
06-062	CC	-/-	339.3	14	12	90	8	58
06-063	CT	-/-	411.9	12	10	93	7	60
06-064	CT	-/-	411.6	10	8	115	8	62
06-065	CC	-/-	426.6	5	5	107	8	64
06-066	CC	-/-	318.7	12	8	79	7	60
06-067	CC	-/-	388.3	7	5	98	8	64
06-068	CC	-/-	387.1	11	8	88	8	60
06-069	CC	-/-	360.2	4	4	97	8	64
06-070	CT	-/-	444.8	15	14	93	.	C
06-071	CC	-/-	398.3	13	12	96	7	58
06-072	CT	-/+	359.9	10	8	83	7	62
06-073	CT	-/-	386.9	7	5	109	7	64
06-074	CC	-/-	364.9	7	5	95	8	64

Lab #	*POMC*	Deletion	HCW (kg)	Average fat	Grade fat	REA (cm^2)	Marbling	Cutability
06-075	CC	-/-	369.2	12	10	91	8	61
06-076	CC	-/-	367.6	5	3	114	8	65
06-077	CT	-/-	362.7	7	6	109	8	64
06-078	CC	-/-	327.5	13	10	91	8	60
06-079	CT	-/-	365.4	5	4	112	7	64
06-080	CC	-/-	353.8	16	12	102	8	59
06-082	CT	-/-	377.4	12	9	93	8	60
06-083	TT	-/-	361.5	7	6	113	8	64
06-084	CT	-/-	348.1	11	9	85	7	62
06-085	TT	-/-	355.8	10	8	105	8	62
06-086	CC	-/-	332.5	5	4	99	9	63
06-087	CT	-/-	420.9	15	16	98	7	54
06-088	CT	-/-	354.5	6	5	115	8	65
06-089	CC	-/-	329.8	4	2	108	9	65
06-090	CC	-/-	353.6	5	4	101	9	64
06-091	CC	-/-	420.0	16	13	92	7	57
06-092	CT	-/-	412.1	7	4	121	8	64
06-093	TT	-/+	383.5	16	12	83	7	57
06-094	CT	-/-	384.0	7	5	114	8	64
06-095	CT	-/+	398.7	13	12	106	7	58
06-097	CC	-/-	444.8	11	9	116	8	61
06-098	CC	-/-	344.3	4	3	101	8	65
06-100	CC	-/-	365.6	11	7	107	7	64
06-101	CC	-/-	374.7	5	4	125	9	64
06-102	CT	-/-	386.7	7	4	123	8	62
06-103	CC	-/-	382.2	10	10	93	7	58
06-104	CC	-/-	350.6	11	10	91	8	59
06-105	TT	-/-	421.4	11	9	117	8	64
06-106	CT	-/-	392.1	5	4	128	8	64
06-107	CC	-/-	362.7	9	8	91	7	61
06-108	CC	-/-	404.6	12	11	87	8	57
06-109	CC	-/-	361.3	10	8	98	8	62
06-110	CT	-/-	392.8	14	10	89	8	60
06-111	CT	-/+	404.4	11	10	92	7	60
06-112	CT	-/-	349.0	7	5	117	8	64
06-113	CC	-/-	373.8	11	11	87	8	58
06-114	CT	-/-	365.4	8	6	112	8	64
06-115	CT	-/-	395.2	10	8	86	7	60

Lab #	*POMC*	Deletion	HCW (kg)	Average fat	Grade fat	REA (cm^2)	Marbling	Cutability
06-116	CC	-/-	372.9	8	7	104	8	64
06-117	CT	-/-	377.2	7	5	116	8	64
06-118	CC	-/-	375.4	8	7	105	8	64
06-119	CC	-/-	383.7	9	7	101	8	64
06-120	CC	-/-	357.0	14	13	96	7	58
06-121	CC	-/-	379.7	9	7	119	7	62
06-122	CC	-/-	350.4	5	5	96	8	62
06-123	CC	-/-	385.6	14	12	84	7	57
06-124	CC	-/-	325.9	11	8	97	8	61
06-125	CC	-/-	346.1	9	8	82	8	59
06-126	CC	-/-	391.2	9	8	107	8	61
06-127	CT	-/-	396.4	8	6	105	8	62
06-128	CC	-/-	348.1	11	9	97	8	60
06-129	CC	-/-	382.4	13	10	89	8	58
06-130	CT	-/-	338.4	7	5	104	9	64
06-131	CC	-/-	379.4	14	12	81	8	56
06-132	CT	-/-	318.9	5	4	96	8	64
06-133	CC	-/-	362.7	11	9	93	8	61
06-134	CC	-/-	338.8	13	12	97	8	59
06-136	TT	-/-	371.7	8	7	110	7	64
06-137	CT	-/-	353.8	7	8	107	8	62
06-138	CC	-/-	399.8	12	13	101	8	58
06-139	CC	-/-	366.1	9	7	96	7	62
06-140	CC	-/-	391.5	17	15	103	7	55
06-141	CC	-/-	352.4	9	8	101	7	60
06-142	CC	-/-	392.4	9	7	94	7	61
06-143	CC	-/-	377.6	11	10	88	7	58
06-144	CC	-/-	376.9	10	8	89	7	60
06-145	CC	-/-	371.0	9	7	93	7	64
06-146	CC	-/-	387.4	14	15	87	7	55
06-147	CC	-/-	378.8	8	6	106	8	64
06-148	CT	-/-	374.4	9	7	106	7	62
06-149	CC	-/-	360.2	9	7	99	8	61
06-150	CC	-/-	396.1	7	5	96	8	64
06-151	CC	-/-	389.4	12	10	102	7	59
06-152	CC	-/-	371.3	14	12	88	7	58
06-153	TT	-/-	406.4	12	10	79	8	56
06-154	CC	-/-	359.7	7	5	109	8	64

Lab #	*POMC*	Deletion	HCW (kg)	Average fat	Grade fat	REA (cm^2)	Marbling	Cutability
06-155	CT	-/-	349.3	6	5	97	8	61
06-156	CC	-/-	397.4	12	9	94	8	61
06-157	CT	-/-	392.4	9	7	115	9	64
06-158	CC	-/-	341.6	4	3	108	7	65
06-159	CT	-/-	366.5	15	15	82	8	54
06-160	CT	-/-	433.2	9	6	107	8	64
06-161	TT	-/-	404.4	6	5	112	8	64
06-162	CC	-/-	433.6	10	9	109	8	64
06-163	CT	-/-	451.1	11	7	114	8	62
06-164	CC	-/-	361.0	8	7	92	8	62
06-165	CT	-/-	325.5	4	3	98	8	64
06-166	CC	-/-	387.6	6	5	118	8	64
06-167	CT	-/-	376.5	5	4	102	8	64
06-168	CT	-/+	341.6	13	12	83	8	57
06-169	TT	-/-	334.8	8	6	84	8	62
06-170	CT	-/-	421.2	19	17	94	7	54
06-171	CC	-/-	385.6	6	5	104	8	64
06-172	CT	-/-	336.1	2	1	117	.	MF
06-173	TT	-/-	365.8	10	7	104	8	62
06-174	CC	-/-	381.0	6	5	107	8	64
06-175	CC	-/-	341.6	5	4	116	8	65
06-176	CC	-/-	358.1	6	4	85	7	64
06-177	TT	-/-	331.8	13	12	96	8	58
06-178	CT	-/-	398.7	5	4	96	7	64
06-179	CC	-/-	378.3	10	7	92	8	63
06-180	CC	-/-	320.7	8	7	87	8	62
06-181	CT	-/-	350.2	11	9	94	8	58
06-182	CC	-/-	363.8	9	8	86	8	60
06-183	TT	-/-	360.2	16	17	78	8	53
06-184	CT	-/-	381.9	10	11	84	8	57
06-185	CT	-/-	432.1	8	7	110	8	64
06-186	CT	-/-	411.4	8	6	118	8	64
06-187	CC	-/-	406.0	15	16	91	7	55
06-188	CC	-/-	387.6	5	4	123	8	64
06-189	CC	-/-	394.6	10	7	106	7	64
06-190	CT	-/-	388.7	8	6	104	8	62
06-191	CC	-/-	384.0	6	5	99	8	64
06-192	CT	-/-	432.1	10	9	93	7	60

Lab #	*POMC*	Deletion	HCW (kg)	Average fat	Grade fat	REA (cm^2)	Marbling	Cutability
06-193	CC	-/-	395.1	15	12	93	8	58
06-194	CC	-/-	342.2	7	5	98	8	64
06-195	CC	-/-	373.8	9	6	99	8	63
06-196	CT	-/-	372.9	10	8	113	8	62
06-197	CT	-/-	387.8	5	5	129	9	64
06-198	CC	-/-	377.4	8	7	103	7	62
06-199	CT	-/-	350.2	16	15	89	8	55
06-200	CC	-/-	373.3	10	8	124	7	64
06-201	CT	-/+	400.1	16	14	91	8	57
06-202	CC	-/-	366.5	6	5	121	8	64
06-203	CC	-/-	395.5	12	10	115	8	61
06-205	CT	-/-	362.9	3	3	102	.	C
06-206	CC	-/-	377.8	15	14	87	7	58
06-207	CC	-/-	340.2	12	11	88	7	58
06-208	CC	-/-	350.6	16	15	85	8	55
06-209	CC	-/-	367.4	13	14	97	7	57
06-210	CT	-/-	342.5	9	8	101	8	62
06-211	CT	-/-	368.1	7	5	113	7	64
06-212	CT	-/+	352.0	10	9	91	7	62
06-213	CC	-/-	389.0	10	8	86	7	62
06-214	CT	-/-	422.3	7	5	123	8	64
06-215	CT	-/-	364.9	10	8	81	8	59
06-216	CC	-/-	418.9	6	5	128	8	64
06-217	CC	-/-	365.4	7	6	94	8	61
06-218	CT	-/-	397.8	7	5	125	8	64
06-219	CC	-/-	370.6	14	12	98	8	59
06-220	TT	-/-	334.5	6	5	109	8	64
06-221	CT	-/-	393.5	12	11	92	7	58
06-223	CC	-/-	358.1	11	11	87	8	56
06-224	CC	-/-	408.0	8	7	108	7	62
06-225	CT	-/-	398.0	3	2	125	9	65
06-226	CC	-/-	406.2	12	9	106	7	62
06-227	CC	-/-	388.7	4	4	115	8	64
06-228	TT	-/-	377.8	6	5	97	8	62
06-230	CC	-/-	367.0	7	5	116	.	C
06-231	CC	-/-	390.8	14	15	104	7	58
06-232	CT	-/-	345.6	9	7	99	7	62
06-233	CC	-/-	415.5	20	18	123	7	54

Lab #	*POMC*	Deletion	HCW (kg)	Average fat	Grade fat	REA (cm^2)	Marbling	Cutability
06-234	CC	-/-	359.0	16	15	83	7	55
06-235	CC	-/-	375.4	9	8	103	7	61
06-236	CC	-/-	422.1	9	7	103	7	64
06-237	CC	-/-	359.8	9	8	91	8	63
06-238	CT	-/-	386.2	10	9	98	8	62
06-240	CT	-/-	401.9	14	12	96	8	58
06-241	CC	-/-	389.9	11	9	102	7	59
06-242	CC	-/-	388.1	19	19	87	7	53
06-243	CC	-/-	337.0	7	6	94	8	63
06-244	CT	-/-	373.8	15	13	88	7	57
06-245	CT	-/-	400.8	13	13	107	7	58
06-246	CC	-/-	392.2	12	12	93	7	57
06-247	CC	-/-	359.7	13	12	98	8	58
06-248	CC	-/-	365.4	14	13	96	7	58
06-250	CC	-/-	339.3	6	5	87	8	64
06-251	CC	-/-	376.9	14	13	99	7	57
06-252	CT	-/-	368.5	13	13	101	8	58
06-253	CT	-/-	374.7	12	10	79	7	61
06-254	CT	-/+	358.1	11	9	99	7	52
06-255	CC	-/-	366.1	9	8	92	8	60
06-256	CC	-/-	408.0	16	14	96	7	54
06-257	CT	-/-	330.7	11	9	107	.	C
06-258	CT	-/-	402.8	8	5	105	8	62
06-259	CC	-/-	386.5	10	9	97	8	62
06-260	CC	-/-	361.1	9	7	104	8	62
06-261	CC	-/-	386.9	9	8	111	8	62
06-262	CT	-/-	422.5	12	10	114	8	61
06-263	CT	-/-	415.0	14	11	113	7	59
06-264	CT	-/-	423.0	16	14	96	7	55
06-265	CC	-/-	353.4	13	8	95	7	60
06-266	CT	-/-	366.7	4	4	121	9	65
06-267	CC	-/-	385.8	10	8	114	8	61
06-268	CC	-/-	389.0	8	7	122	8	62
06-269	CT	-/-	397.1	8	7	131	8	64
06-270	TT	-/-	391.9	9	8	103	7	62
06-271	CC	-/-	370.4	4	3	117	9	64
06-272	CT	-/-	424.8	8	5	118	8	64
06-273	CC	-/-	373.5	10	10	104	8	59

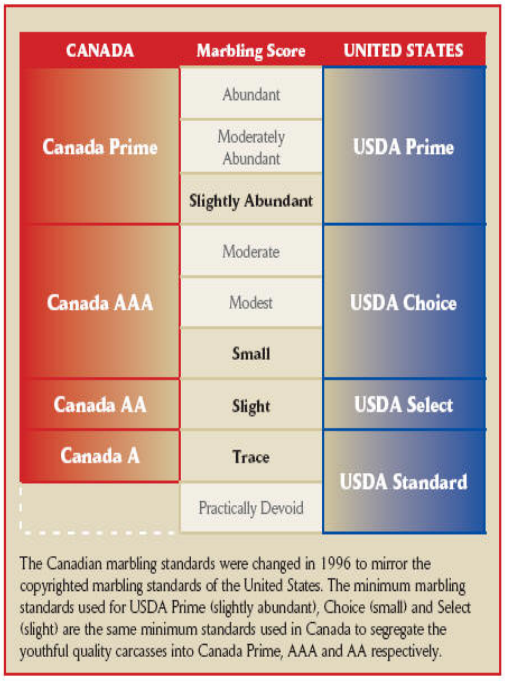
Lab #	*POMC*	Deletion	HCW (kg)	Average fat	Grade fat	REA (cm^2)	Marbling	Cutability
06-274	CC	-/-	344.1	9	7	87	7	61
06-275	CC	-/-	383.5	13	10	99	8	61
06-276	TT	-/-	423.9	7	6	117	.	C
06-277	CC	-/-	396.0	11	8	108	7	62
06-278	CT	-/-	416.9	9	7	117	8	62
06-279	CC	-/-	350.6	9	7	96	8	64
06-280	CT	-/-	359.2	5	4	128	8	64
06-282	TT	-/-	394.2	13	11	101	8	61
06-283	CT	-/-	377.6	14	12	91	7	58
06-284	CT	-/-	387.8	7	8	99	8	60
06-285	CC	-/-	386.2	16	16	83	7	54
06-286	CT	-/-	361.1	10	8	105	8	62
06-287	CT	-/-	383.3	10	9	106	8	61
06-288	CT	-/-	393.7	19	18	95	8	54
06-289	CT	-/-	415.9	9	7	129	8	64
06-290	CT	-/-	394.0	10	8	115	8	61
06-291	CT	-/-	413.2	10	7	117	8	64
06-292	CT	-/-	417.8	8	6	109	8	64
06-293	CC	-/-	383.1	9	7	115	7	62
06-294	CT	-/-	403.0	11	11	114	8	61
06-295	CT	-/-	372.9	9	8	109	8	64
06-296	CT	-/-	399.8	6	6	127	9	64
06-297	CC	-/-	388.3	14	11	97	7	60
06-298	CC	-/-	317.5	7	4	84	8	63
06-299	CC	-/-	348.8	12	12	83	7	57
06-300	CT	-/-	373.8	13	11	94	8	58
06-301	CT	-/-	345.1	17	15	87	7	57
06-302	CC	-/-	328.4	10	7	83	7	61
06-303	CC	-/-	377.8	14	14	95	8	58
06-304	CT	-/+	347.7	9	8	96	7	62
06-305	CC	-/-	399.2	13	13	88	8	57
06-306	CT	-/-	373.8	12	11	97	8	59
06-307	CC	-/-	380.1	14	10	94	8	58
06-308	CC	-/-	342.0	8	8	99	8	62
06-309	CC	-/-	376.0	12	10	97	8	59
06-310	CT	-/-	372.9	13	12	83	8	60
06-311	CT	-/-	385.3	15	13	80	7	54
06-312	TT	-/-	340.7	5	4	98	9	64

Lab #	*POMC*	Deletion	HCW (kg)	Average fat	Grade fat	REA (cm^2)	Marbling	Cutability
06-313	CC	-/-	367.4	12	12	97	7	58
06-314	CC	-/-	335.7	6	4	94	8	64
06-315	CC	-/-	372.2	15	14	91	8	57
06-316	CC	-/-	389.0	16	16	90	7	56
06-318	CC	-/-	333.4	8	7	104	8	62
06-319	CC	-/-	368.8	6	5	93	7	64
06-320	CC	-/-	342.2	11	8	107	8	62
06-321	CC	-/-	446.8	16	16	111	7	56
06-322	CC	-/-	383.3	13	10	105	8	59
06-323	CC	-/-	430.7	14	12	96	7	59
06-324	CC	-/-	415.3	13	11	110	7	59
06-325	CC	-/-	323.9	7	5	96	8	64
06-326	CC	-/-	392.6	6	5	115	7	64
06-327	CC	-/-	337.9	9	7	96	9	64
06-328	CC	-/-	353.6	13	12	89	7	60
06-329	CC	-/-	364.0	11	8	94	8	62
06-330	CT	-/-	401.0	6	5	116	8	64
06-331	CT	-/-	323.2	13	9	89	8	61
06-332	CT	-/-	408.2	14	13	96	7	57
06-333	CC	-/-	379.0	3	3	118	8	65
06-334	CC	-/-	403.7	4	4	131	8	64
06-335	CT	-/-	384.2	8	7	107	8	64
06-336	CC	-/-	386.5	10	8	104	8	62
06-337	CC	-/-	383.3	10	8	114	7	64
06-338	CT	-/-	371.0	12	9	91	8	58
06-339	CT	-/-	382.2	12	10	107	8	61
06-340	CT	-/-	419.6	16	14	123	7	58
06-341	CC	-/-	386.1	9	9	101	8	60
06-342	CT	-/-	365.8	2	2	123	9	65
06-343	CC	-/-	380.8	7	7	93	8	64
06-344	CC	-/-	362.9	4	4	119	8	64
06-345	CC	-/-	383.5	4	2	131	9	65
06-346	CC	-/-	371.7	12	10	112	7	61
06-347	CC	-/-	388.7	10	8	96	8	62
06-348	CC	-/-	369.5	12	10	93	8	61
06-349	CC	-/-	348.4	7	5	101	7	62
06-350	CT	-/-	383.3	10	8	97	8	62
06-351	CC	-/-	401.2	9	8	99	8	62

Lab #	*POMC*	Deletion	HCW (kg)	Average fat	Grade fat	REA (cm^2)	Marbling	Cutability
06-352	CC	-/-	372.6	13	11	107	8	58
06-353	TT	-/-	380.3	6	6	116	8	64
06-354	CC	-/-	418.2	12	9	108	7	62
06-355	CT	-/-	357.2	12	9	104	8	61
06-356	CT	-/-	462.4	5	4	123	8	64
06-357	CC	-/-	393.5	6	5	128	8	64
06-358	CT	-/-	381.2	11	10	99	8	61
06-359	TT	-/-	392.4	6	5	123	8	65
06-360	CT	-/-	352.4	9	7	103	8	61
06-361	CC	-/-	317.5	5	4	95	9	65
06-362	CT	-/-	370.1	9	7	94	7	62
06-363	CT	-/-	316.4	9	7	97	8	63
06-364	CC	-/-	371.7	17	16	86	7	57
06-365	CT	-/-	347.0	9	7	107	9	60
06-366	CC	-/-	420.9	8	9	110	.	C
06-367	CT	-/-	329.3	10	7	77	8	62
06-368	CT	-/-	380.6	5	5	114	8	65
06-369	CC	-/-	382.6	11	9	107	7	60
06-370	CT	-/+	317.1	8	6	94	8	62
06-371	CT	-/-	379.9	9	7	104	8	64
06-372	CT	-/-	378.8	16	16	76	7	52
06-373	CC	-/-	411.4	9	7	109	8	62
06-374	CT	-/-	371.3	12	9	108	8	61
06-375	CC	-/-	329.8	13	11	97	7	58
06-376	CT	-/-	375.4	4	4	109	8	65
06-377	CC	-/-	365.4	24	23	93	7	52
06-378	CT	-/-	413.7	15	12	114	8	58
06-379	CT	-/-	306.2	5	4	88	.	C
06-380	CC	-/-	347.5	11	10	102	8	61
06-381	CC	-/-	388.1	12	12	96	8	60
06-382	CC	-/-	352.4	12	11	97	8	58
06-383	CC	-/-	368.3	10	8	113	8	62
06-384	CC	-/-	349.3	5	4	117	8	64
06-385	TT	-/-	388.5	8	7	95	7	64
06-386	CC	-/-	390.5	12	10	101	7	59
06-387	CC	-/-	405.5	7	5	122	8	64
06-388	CC	-/-	324.1	6	5	108	8	64
06-389	CC	-/-	339.5	9	7	105	8	64

Lab #	*POMC*	Deletion	HCW (kg)	Average fat	Grade fat	REA (cm^2)	Marbling	Cutability
06-390	CC	-/-	372.6	7	5	104	8	64
06-391	TT	-/-	392.1	8	7	109	8	62
06-392	CT	-/-	394.6	12	10	106	7	61
06-393	CC	-/-	376.3	13	13	87	7	56
06-394	CC	-/-	360.2	6	5	106	8	64
06-395	CC	-/-	320.9	10	7	79	7	61
06-396	CC	-/-	376.5	7	6	101	8	64
06-397	CC	-/-	408.2	9	8	109	8	64
06-398	CC	-/-	365.1	9	9	114	8	62
06-399	CC	-/-	314.1	4	4	112	8	64
06-400	CC	-/-	335.9	3	2	112	8	65
06-401	CT	-/-	338.6	9	8	93	8	62

Appendix B Carcass Grading



Marbling score was conducted at XL Beef in Moose Jaw using a scale of 2-10 with 2 being abundant and 10 being practically devoid (Based on information received May 25, 2009 from Tom Chappell. Meat Grader, Moose Jaw).

Appendix C Test for Hardy Weinberg Equilibrium of the deletion genotypes

The expected values were calculated using the minor allele frequency of 0.02 in relation to the 386 cattle genotyped.

2 x 3 contingency table:

	+/+	+/-	-/-
Observed	372	14	0
Expected	372	14	0

Since there were values of 0 in the -/- cells, the appropriate statistical test for the Hardy Weinberg Equilibrium would be the Fisher exact test using the formula below. However, since the observed and expected values were identical, there was no need to run this statistic.

Fisher exact test formula:

$$P = \frac{(A+B+C)!\,(D+E+F)!\,(A+D)!\,(B+E)!\,(C+F)!}{N!\,A!\,B!\,C!\,D!\,E!\,F!}$$

Appendix D Genbank Submission

LOCUS bankit1233517 931 bp DNA linear MAM 17-JUN-2009
DEFINITION Bos taurus pro-opiomelanocortin (*POMC*) gene, exon 3 and partial cds.
ACCESSION 1233517
VERSION
KEYWORDS
SOURCE Bos taurus
ORGANISM Bos taurus
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
Pecora; Bovidae; Bovinae; Bos.
REFERENCE 1 (bases 1 to 931)
AUTHORS Deobald,H.M., Buchanan,F.C. and Marquess,F.L.
TITLE Effects of a 12 bp deletion in *POMC* on carcass characteristics in beef
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 931)
AUTHORS Deobald,H.M. and Buchanan,F.C.
TITLE Direct Submission
JOURNAL Submitted (17-JUN-2009) Animal and Poultry Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada
COMMENT Bankit Comment: fiona.buchanan@usask.ca.
FEATURES Location/Qualifiers
source 1..931
/organism="Bos taurus"
/mol_type="genomic DNA"
/db_xref="taxon:9913"
/chromosome="11"
/sex="female"
/breed="Angus"
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CDS <176..841
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/note="*POMC* exon 3"
/codon_start=1
/product="Pro-opiomelanocortin"

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AAARAELEYGLVAEAEAEAAEKKDSGPYKMEHFRWGSPPKDKRYGGFMTSEKSQTPLVTLFKNAIIKNAHKKGQ"

variation 331
/gene="*POMC*"
/note=C/T heterozygous position"
variation 336..347
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/note="heterozygous for 12 bp deletion TTGGGGGCGCGG
4 amino acids removed VGGA
3'UTR 842..931
/note="842-931"
BASE COUNT 170 a 290 c 348 g 123 t

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121 tgaacgccgc ggcctggagt gggcggggcc tgacgggctc gccgctctcc cgcaggcgtg
181 catccgggcc tgcaagcccg acctctccgc cgagacgccg gtgttccccg gcaacggcga
241 tgagcagccg ctgactgaga acccccggaa gtacgtcatg ggccatttcc gctgggaccg
301 cttcggccgt cggaatggta gcagcagcag cggagttggg ggcgcggccc agaagcgcga
361 ggaggaagtg gcggtgggcg aaggccccgg gccccgcggc gatgacgccg agacgggtcc
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901 ccctcctacc gcctcgcagc ctgggtgagg a

Appendix E ISAG Abstract

International Society for Animal Genetics XXXI, July 20-24, 2008, Amsterdam, The Netherlands.

Characterization of a deletion in the cattle Pro-opiomelanocortin gene.

Heather M. Deobald, Sheila M. Schmutz and Fiona C. Buchanan.

Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada.

Pro-opiomelanocortin (*POMC*) is a complex gene with four gene products that play roles in the appetite, stress and pigmentation pathways. Previous data suggest that a SNP in *POMC* (*POMC c.288C>T*) is associated with shipping and hot carcass weight in cattle. While optimizing this variant using real time PCR an unexpected shift in melting temperatures was observed. Sequencing these animals revealed a 12 bp deletion (*POMC c.293_304delTTGGGGGCGCGG*) that results in four amino acids being removed (Val, Gly, Gly, Ala), and occurs five nucleotides downstream from the original SNP. The deletion starts at the second position of the 98th codon however it does not cause a frame shift. The allele frequency of the deletion was 2% in 386 steers and was only observed with the T allele. No homozygous deletion animals were found. A Mann Whitney U test showed an association with the presence or absence of the deletion and ultrasound rib-eye area at end of backgrounding (P= 0.036) and carcass rib-eye area (P=0.028).

Appendix F ISAG Poster

2089

Characterization of a Deletion in the Cattle Pro-opiomelanocortin Gene.

Heather M. Deobald, Sheila M. Schmutz and Fiona C. Buchanan

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Introduction

Pro-opiomelanocortin (POMC) is a complex gene with numerous peptide products (Fig. 1, Pritchard et al. 2000), some of which play a role in the appetite, stress and pigmentation pathways. Namely, alpha melanocyte stimulating hormone (α-MSH) and β–MSH act as ligands for the melanocortin-4 receptor (MC4R; Marsh et al. 1999, Harrold et al. 2003), which when bound reduce appetite. α-MSH also binds to MC1R where it affects coat colour (Klungland et al. 1995). Adrenocorticotropic hormone (ACTH) is an intermediate product in the stress response pathway that is controlled by the hypothalamus pituitary adrenal axis. A single nucleotide polymorphism (SNP) in *POMC* c.288C>T was used to map this gene to chromosome 11 in cattle where a QTL for carcass weight and average daily gain had been identified (Buchanan and Thue, 2003). Subsequently an association with shipping and hot carcass weights as well as average daily gain was reported (Buchanan et al. 2005). While optimizing this variant for high throughput genotyping using real time PCR, an unexpected shift in melting temperatures was observed (Fig. 2).

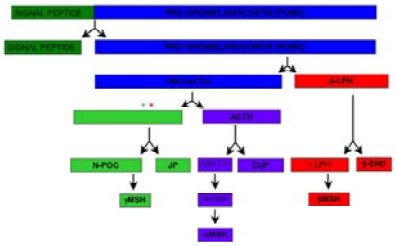

Figure 1. The *POMC* processing pathway in the hypothalamus. The green * and red * indicate the approximate positions of the SNP and deletion respectively (Modified from Pritchard et al. 2002).

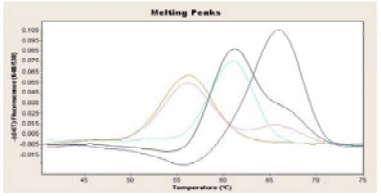

Figure 2. Melting temperature peaks from the LightCycler 2.0. Tm at 61°C denotes T allele, Tm at 66°C denotes C allele. Tm at 56°C represent abnormal melting curves from the POMC c.288C>T genotyping assay.

Material and Methods

Initially five animals exhibited the shift in melting temperature in the real time PCR assay. These were sequenced. Exon 3 of *POMC* was amplified and genotyped for the SNP (Buchanan and Thue 2003). The following primers (POMCdelF = CCGTCGGAATGGTAGCA and POMCdelR = CGTTGGGGTACACCTTC) amplified a 206 bp fragment of exon 3 that was then electrophoresed on a 4% agarose gel to detect the 12 bp deletion (Fig. 3). A Mann Whitney U test was performed to look at the effect of the deletion on various carcass traits.

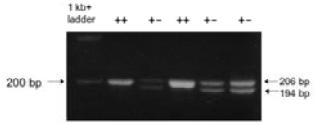

Figure 3. A 4% agarose gel displaying the wildtype (+) or the 12bp deletion (-) in the *POMC* gene from crossbred steer genomic DNA.

Results and Discussion

Direct sequencing identified a 12 bp deletion (*POMC* c.293_304delTTGGGGGCGCGG) that results in four amino acids being removed (Val, Gly, Gly, Ala) (Fig. 4). The deletion occurs 5 nucleotides downstream from the original SNP, *POMC* c.288C>T. No frame shift occurs as a result of the deletion. The allele frequency of the deletion was 2% and it was only observed with the T allele. The allele frequency of *POMC* c.288C>T was 0.26 and 0.74 for the T and C alleles respectively. A Mann Whitney U test showed a significance of P = 0.036 with ultrasound rib-eye area at end of back grounding, and P = 0.028 with carcass rib eye area.

a)

AGC GGA GTT GGG GGC GCG GCC CAG AAG CGC GAG GAG
S G V G G A A Q K R E E

b)

AGC GGA GCC CAG AAG CGC GAG GAG
S G A Q K R E E

Figure 4. Partial sequence from exon three of *POMC*. a) highlighted in red are the twelve deleted base pairs and the affected amino acids b) the same sequence minus the 12 bps and 4 amino acids, the first position in the codon for valine and the 2nd and 3rd positions of the second alanine are coloured in blue. The *POMC* c.288C>T SNP is shown in green.

Conclusions

- From an evolutionary stand point, the SNP occurred first with the deletion occurring more recently.
- It is likely that the homozygous deletion is lethal.
- The deletion was also associated with a 7.04 cm^3 decrease in carcass rib eye area.

Summary

Dependent on the frequency of the deletion in specific breeds, it may be beneficial to select against the deletion.

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Acknowledgments

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