Impact of Genetics on Meat Quality of Pigs and Beef Cattle

by

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Abstract

Meat has changed its role from just providing necessary nutrition for the human body to improving the quality of life by giving us eating satisfaction, resulting in the impetus for scientific research on meat quality. Although many strategies have been taken to improve meat quality, unacceptably inferior meat still exists, causing economic loss for the meat industry. Potentially, improving meat quality through animal breeding offers opportunities to obtain superior meat. Hence, to explore the possibility of future genetic selection of animals for meat quality, several studies investigating different meat quality traits, different species, and different sample handling strategies were conducted.

Dark cutting beef is a significant defect caused by depletion of muscle glycogen before slaughter that may be affected by animal genetics. A case-control genome-wide association study (GWAS) on two groups of beef cattle was conducted and dark-cutting was analyzed as a binary trait (cases versus controls) using logistic regression in an additive model. There were no significant loci identified when correcting for multiple testing (false discovery rate, FDR) using a FDR < 0.05 threshold. The regions with the strongest support for association with the occurrence of dark cutting were identified using a 1 MB window and functional analysis using the Ingenuity Pathway Analysis (IPA), which identified genes involved in pyruvic acid modification, 2-deoxyglucose clearance and disposal, pyruvic acid release, sucrose recognition, energy production and metabolism of carbohydrate. Although the detected SNP associations require validation, results suggested the possibility for marker-assisted genomic selection of beef cattle for reduced likelihood of dark cutting; however, based on these results a much larger number of case samples will be required to validate these observations

Consumer willingness to pay a premium when purchasing pork chops is driven by eating satisfaction. Genetic parameters were estimated for loin muscle sensory traits within a swine population and their associations with loin pH and intramuscular fat were analyzed. Animal pedigree and genotype information were analyzed separately, and positive genetic correlations between sensory measurements and pH and intramuscular fat were found, indicating that selection for intermediate pH and high intramuscular fat can help to increase sensory scores. However, as the genetic correlations were moderate to low, increase in pork sensory scores through selection for loin pH and intramuscular fat content would be slow.

Important meat quality characteristics have been measured on fresh and previously frozen meat as part of previous genetic studies, but freezing may alter meat quality characteristics and therefore the relationships between genetic components and meat quality measurements. Results showed that pork quality traits measured before and after freezing and thawing were significantly ($P<0.0001$) different from each other and that intramuscular crude fat content exerted a large effect on the magnitude of change in L* (lightness) and b* (yellowness). Meat quality measurements of fresh pork were moderately to highly heritable except for b* and pH, with heritability estimates for L*, pH and drip loss higher when measured on fresh rather than frozen-thawed samples. Considering heritability and genetic correlation results, it could be concluded that whilst either fresh or frozen-thawed pork samples can be used for L*, a* (redness) and b* measurements can be used in genetic selection, pH and possibly drip loss should be measured in fresh pork samples rather than in those that have been frozen-thawed.

Tenderness is one of the most important factors considered by consumers when purchasing meat and intramuscular connective tissue (IMCT) is a major factor responsible for the cooked meat background toughness. A GWAS was designed to identify variations (e.g.:

single nucleotide polymorphisms (SNPs)) in genes along the genome associated with total collagen and collagen solubility. In total, 130 SNPs were detected for 3-day postmortem (3 dpm) total collagen content using SNP windows that explained more than 1% of the additive genetic variance, while 160 SNPs were detected for 3 dpm collagen solubility, and 150 and 190 SNPs were detected for 13 dpm total collagen content and collagen solubility, respectively. These results should be validated in a large beef cattle group before considering marker-assisted or genomic selection in beef cattle to increase beef tenderness. Collectively these results indicated that selection against dark cutting and for increased collagen solubility in beef, and for increased pork acceptability may be possible.

Preface

Along with the development of society, the role of meat has changed from just providing necessary nutrition for the human body to improving the quality of life by giving us eating satisfaction, leading to comprehensive scientific research in the meat quality area. Regardless of the many strategies being taken to improve meat quality, unacceptable meat with inferior quality persists, causing economic loss for the meat industry. Potentially, improving meat quality through animal breeding may lead to superior meat. This thesis incorporated several studies to explore the possibility of future genetic selection of animals to produce better meat.

In Chapter 2 (A genome-wide case-control association study of dark cutting in beef cattle), two groups of beef cattle were used for genome-wide association study (GWAS) to identify variations (e.g.: SNPs) in genes associated with dark cutting beef and explore the biological relevance of those genes in the formation of dark cutting beef. This chapter has been submitted to Canadian Journal of Animal Science where Dr. Tianfu Yang, Dr. Shahid Mahmood, Dr. Bimol C. Roy, Dr. Changxi Li, Dr. Graham S. Plastow, and Dr. Heather L. Bruce are co-authors.

For Chapter 3 (Genetic parameter estimation for sensory traits in longissimus muscle and their association with pH and intramuscular fat in pork chops), genetic parameters, including heritability, genetic and phenotypic correlations, were estimated for sensory traits of the longissimus muscle from 784 crossbred commercial pigs. The influence of intramuscular fat content and pH on pork chop sensory acceptability was also discussed. The sensory panel was run by Dr. Chamali Das. The data were analyzed using animal pedigree information and genotype information separately, and their difference was discussed.

For Chapter 4 (Efficacy of genetic parameter estimation of pork loin quality of crossbred commercial pigs using technological quality measurements of frozen and unfrozen product), fresh and frozen-thawed meat samples from more than 2000 crossbred commercial pigs were used to estimate genetic parameters, such as heritability, and genetic and phenotypic correlations between fresh and frozen-thawed products. This study reached a conclusion that either fresh or frozen-thawed samples could be used for L*, a* and b* measurements, but pH and drip loss should be measured in fresh samples rather than in frozen-thawed ones for genetic selection. Chapter 4 has been published in the Canadian Journal of Animal Science where Dr. Chunyan Zhang, Dr. Changxi Li, Dr. Graham S. Plastow, and Dr. Heather L. Bruce are co-authors (Canadian Journal of Animal Science, 2018, 98(3): 453-462, https://doi.org/10.1139/cjas-2017-0154).

Chapter 5 (Genome-wide association study of collagen in beef cattle) aims to identify variation (e.g. SNPs) in genes along the genome associated with total collagen and collagen solubility, and to explore the biological relevance of the genes to beef toughness caused by intramuscular connective tissue. In total, 137 beef cattle *raised and managed at* the *Roy Berg Kinsella Ranch, University of Alberta, Canada,* were used in this study. Single-step Genomic Best Linear Unbiased Prediction (ssGBLUP) was used for GWAS and significant results (SNP windows that explained more than 1% additive genetic variance) were obtained.

Huaigang Lei

To my wife, Tao Wang

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1. General Introduction

Meat, rich in water, protein, and fat, is a good source of proteins obtained from animal flesh for human consumption. Hunting and killing animals for their meat can be dated back to 8,000-10,000 years before present, when the history of domestication began (Bruford et al. 2003). In the modern world, meat has not only become the main protein food source, but also become essential to the diet of our everyday lives, leading to a flourishing meat industry. With the development of our society, the role of meat has changed from just providing necessary nutrition for the human body to improving the quality of life as well through eating satisfaction, resulting in comprehensive scientific research in meat quality.

1.1. Meat quality attributes

Meat can only provide us with nutrients after it is consumed, which gives importance to the pleasure associated with its taste and appearance (Pearson 2013). Generally, meat quality includes four aspects: the security of the meat, which describes the hygienic quality of the product; the healthfulness of the meat, which includes all the nutritional benefits of consuming meat; serviceability of the meat, which focuses on what a consumer perceives as the functionality of the product, like ease of use, ability to be processed, and price; and the satisfaction with meat characteristics such as its color, texture, juiciness and flavor (Listrat et al. 2016). Nowadays, the satisfaction of purchasing and eating meat attracts increasing attention given that consumer satisfaction is the prerequisite of continuous consumption and consumer loyalty (Bearden and Teel 1983). The most important meat quality traits directly or indirectly related to consumer satisfaction include meat pH, colour, tenderness, juiciness and flavor.

1.1.1. Meat pH

The measurement of meat pH was first introduced in pork research to diagnose possible pale, soft and exudative (PSE) carcasses (Wismer-Pedersen 1959) and in 1991 ultimate pH value

was recognized as a primary postmortem factor influencing pork quality (Offer 1991). After being slaughtered, hydrogen ions in an animal's skeletal muscle will accumulate mainly through muscle anaerobic glycolysis, causing the muscle pH to decrease (Bendall 1973). During postmortem aging, intramuscular pH will decrease gradually from about 7.2 to 5.6 (ultimate pH) and the rate and extent of pH decline has an important influence on final meat quality attributes (Matarneh et al. 2017). As muscle pH declines postmortem, a zero net charge of the muscle proteins will be reached when the muscle pH approaches the isoelectric point of the muscle proteins. Without any charges, proteins inside the muscle will be attracted to each other, reducing the space available for water to reside in the muscle, leading to the decrease of water holding capacity of the muscle. In addition, with reduced like charges, electrostatic repulsion between structures inside the muscle will be limited or eliminated and, as a result, myofilaments will be pulled together tightly, reducing the space available for water to be trapped in the muscle, resulting in purge loss (Matarneh et al. 2017). A normal ultimate muscle pH range of 5.5 to 5.8 is essential for the development of superior meat quality, with very low ultimate pH ($pH < 5.5$) associated with the formation of PSE meat (Wismer-Pedersen 1959), while very high ultimate pH ($pH > 5.8$) is associated with the formation of dark cutting meat (Tarrant 1981).

Different relationships between ultimate pH and meat tenderness have been observed by researchers, with Silva et al. (1999) and Guignot et al. (1994) suggesting a linear relationship, while Jeremiah et al. (1991) and Purchas et al. (1993) proposed a curvilinear relationship between meat pH and tenderness. This disagreement might arise from the complicated physiological and biochemical reactions that occur during the aging of meat, with different pathways contributing to the tenderness of meat. According to Yu and Lee (1986), the enzyme activity of calpains, responsible for the degradation of myofibrillar proteins, is high at elevated

muscle pH (> 6.3), which means muscle with increased pH would be expected to be tender compared with that with reduced pH (pH < 6.3). This also explains why dark cutting meat with pH values greater than 6.3, regardless of its unattractive dark colour, is usually more tender than normal meat (Watanabe et al. 1996). To break down actin-myosin cross-bridges formed during rigor mortis, proteolytic enzymes (mainly lysosomal enzymes) need to be activated. However, for lysosomal enzymes to function well, a low muscle pH is required (Schmaljohann 2006), which emphasizes the importance of postmortem pH decline for meat tenderization.

Another major contributor to the background toughness of meat is intramuscular connective tissue (IMCT), with collagen being the major protein found in IMCT (Purslow 2005). Collagen can be degraded by matrix metalloproteinases endogenous to muscle with their highest enzymatic activity occurring at physiological muscle pH (just above 7) (Tarrant 1989; Galis et al. 1994). In terms of IMCT, high pH is best for meat tenderness to develop; therefore, it is difficult to discern which ultimate pH value is best for cooked meat tenderness. This might be one reason why Jeremiah et al. (1991) and Purchas et al. (1993) obtained curvilinear relationships between meat pH and tenderness, showing minimum tenderness between muscle pH values of 5.8 and 6.2 (Silva et al. 1999). Another important meat quality trait influenced by muscle pH is meat colour, which is mainly determined by the different redox states of the sarcoplasmic heme protein called myoglobin, with oxymyoglobin and carboxymyoglobin producing a bright cherry-red colour, deoxymyoglobin giving a purplish-red colour, and metmyoglobin providing a brown colour to the meat (Livingston and Brown 1981; Suman and Joseph 2013). During aging of meat, the decline of muscle pH will activate the activity of metmyoglobin reductase (responsible for the conversion of metmyoglobin to its ferrous form, deoxymyoglobin), which allows the meat to bloom (Mikkelsen et al. 1999). Additionally, without proper pH decline, the muscle will remain

functional and allow little oxygen to be bound to myoglobin, which generates a low and/or sustained oxygen condition, resulting in red oxymyoglobin being oxidized into brown metmyoglobin (Ledward 1970), and subsequent discoloration of the meat.

1.1.2. Water holding capacity

Water holding capacity (WHC) of the fresh meat (often described as drip loss or purge) is the ability of postmortem muscle to retain water and it plays an important role in meat industry as it can affect both the meat yield and the quality of the final product (Huff-Lonergan and Sosnicki 2002). In skeletal muscle, the most abundant composition is water (about 75%) which is held within the muscle structure and muscle cells, such as, within in the myofibrils, between the myofibrils, between the myofibrils and the cell membrane, between muscle fibers, between muscle bundles (surrounded by perimysium) (Offer and Cousins 1992). Generally, there are three types of water found in skeletal muscle, specifically bound water, entrapped water, and free water. Being a small fraction of the total water found in muscle cells, bound water resides among non-aqueous constituents (e.g.: protein molecules) and has limited mobility and, in addition, bound water is very stable and difficult to drive off with conventional heating (Fennema 1985). Entrapped water (immobilized water) is the water held within the structure of the muscle by steric effects and/or by attraction to the bound water, and it is the most affected type of water during the rigor process and the conversion of muscle to meat (Fennema 1985; Huff-Lonergan and Sosnicki 2002). Free water is held by weak surface forces and is not readily seen in pre-rigor meat and so any forces that damage muscle cellular integrity will lead to the release of this type of water from meat.

Factors affecting meat WHC can be summarized as three categories, net charge effect, steric effect, and genetic effect. The net charge effect is mainly observed during the conversion

of muscle to meat. After slaughter, the muscle pH will decrease as anaerobic glycolysis causes the accumulation of hydrogen ions. As the pH keeps declining, the net charge of the protein will become zero once the pH reaches the isoelectric point of the proteins, leading to the attraction of positive and negative groups within the protein and resulting in the reduction in the amount of water that can be attracted and held by that protein. Besides, some structures in the myofibril can pack more closely together due to the decrease of like charges (Huff-Lonergan and Sosnicki 2002). The steric effect is caused by rigor mortis. During the rigor process, the space in which water resides will be reduced due to the formation of cross-bridges between thick and thin filaments, as well as the shortening of sarcomeres (Huff-Lonergan and Sosnicki 2002). To date, there are two major genes that can cause abnormal postmortem muscle pH decline, specifically the *halothane* gene and the *RN*$^{-}$ gene. Pigs that are homozygous (genotype *nn*) carriers of the halothane gene have limited ability to control calcium release into the sarcoplasm of the muscle cell due to a mutation in the ryanodine receptor/calcium release channel and are susceptible to stress, with death being the most intense reaction (Cassens et al., 1975, Webb et al., 1982; Huff-Lonergan and Sosnicki 2002). Because of the accelerated release of calcium, muscle contraction will occur more rapidly and muscle metabolism will become faster, leading to the increase of the rate of pH decline, and rapid pH decline while the carcass is still warm will denature myofibrillar proteins, resulting in the loss of water from the meat. In swine production, if pigs carry the *RN*$^{-}$ gene, there will be an abnormal accumulation of glycogen in their skeletal muscle antemortem, which will increase the glycolytic potential of their skeletal muscle and lead to the dramatic decline of pH very early postmortem. As a result, muscle with low pH while it is still warm often develops PSE meat (Josell et al. 2000), with tremendous water loss. Worthy of note is that the *RN*$^{-}$ gene is not conserved and is mainly found in animals with a Hampshire background.

There are two commonly use methods (both are gravimetric methods) to assess WHC of fresh meat, specifically the bag method and the tray method. The bag method was introduced by Honikel (1998), in which the meat is cut transversely with the direction of muscle fibre, trimmed of epimysium, fat and bone, weighed, suspended by a metal hook in a closed plastic bag (inflated sufficiently to prevent contact with the meat) for 24 h and then weighed again. The tray method was introduced by Barton-Gade et al. (1994), in which the samples were trimmed of subcutaneous fat, weighed, placed on a stainless steel grid above a tray (samples were spaced so that none of the them touched another) for 48 h, and then weighed again. The difference in weights taken before and after the method indicated the loss of water from the muscle and thereby inferred the ability of the meat to hold water.

1.1.3. Meat colour

Meat colour is one of the most important factors controlling a customer's decision at the point of purchasing meat, as colour is the only meat quality attribute that the consumers can easily evaluate, and a bright red colour is widely used by consumers around the world as an indicator of fresh red meat (Suman and Joseph 2013). If the meat shows discoloration such as a brownish dark colour, it will be discriminated against by consumers and its value will be heavily discounted. According to Smith et al. (2000), in United States alone, more than one billion US dollars have been lost in revenue due to the discounted of abnormal colored meat every year.

The formation of certain meat colours is complicated. Overall, it is related to the muscle pigments, antioxidant potential, muscle fibre structure, the state of muscle proteins, as well as intramuscular fat content (Bekhit and Faustman 2005; Bodas et al. 2007; Faustman et al. 2010, Ponnampalam et al. 2012; Ponnampalam et al. 2017). Among the aforementioned factors, the concentration of pigments in skeletal muscle has a dominant influence on the final meat colour.

There are three kinds of pigments inside the muscle, specifically myoglobin, hemoglobin and cytochrome, with myoglobin serving as the main pigment contributing to the favorable bright red or cherry red colour of the meat (Suman and Joseph 2013). Myoglobin, with an iron binding site inside the molecule, is an oxygen-binding protein found in the skeletal muscle tissue, working as an oxygen carrier to support the proper function of the muscle (Kendrew et al. 1960; Wittenberg 1970). Generally, four forms of myoglobin exist in fresh meat based on the redox state of the iron atom inside the protein and the oxygen and carbon monoxide bonding mode: oxymyoglobin and carboxymyoglobin (red, oxygenated form: ferrous/Fe^{2+}), metmyoglobin (brown, oxidized form: ferric/Fe^{3+}) and deoxymyoglobin (purple, reduced form: ferrous/Fe^{2+}) (Suman and Joseph 2013). Those four types of myoglobin are present in fresh meat concurrently and the colour of the meat is the combination of those myoglobins, and the final colour is determined by which type of myoglobin is dominant. According to Neethling (2016), factors influencing meat colour can be roughly divided into extrinsic (like season, feeding system, ante-mortem stress, storage temperature) and intrinsic (like pH, genetics, species, breed, gender, animal age, muscle fibre type, muscle oxidative and reductive capacity, and lipid oxidation).

1.1.4. Meat tenderness

Research shows that consumers are willing to pay a premium on guaranteed tender meat and tenderness is the primary determinant of meat quality as it is considered the most important palatability attribute of meat (Miller et al. 1995; Mintert et al. 2000). Meat tenderness or toughness is a complicated index of myofibrillar degradation, sarcomere length, proteolytic activity and insolubility of collagen (Bongiorni et al. 2016). The typical eating or chewing pattern of meat can be described by two tightly correlated stages: at the beginning of chewing, intact muscle fascicles will be separated from each other, leaving perimysial sheets still in the gaps between nearby fascicles; and, at a later stage of chewing, complete rupture of the meat will

occur after the break down of the perimysial sheets (Purslow 2005). If the meat is consistently tough, consumers will experience eating dissatisfaction and change their future purchase decision, leading to economic loss within the meat industry. The meat tenderization process starts with muscle fiber proteins being degraded by proteolytic enzymes in situ. With the action of these proteases, the structural proteins, including but not limited to actin, myosin, troponin, tropomyosin, titin, desmin and nebulin, will be partially degraded, resulting in softening of the meat. Detailed information related to those proteins can be found elsewhere (Lana and Zolla 2016). With decades of research, the main proteases responsible for the muscle structural protein degradation are calpains (μ-calpains and m-calpains), cathepsins, proteasomes and caspases (Lana and Zolla 2016). Within these enzymes, calpains (mainly μ-calpains, coded by *CAPN1* gene) are the most extensively studied and are believed to contribute most to the meat tenderization process (Kemp et al. 2010). Calpastatin, coded by *CAST* gene, is the binding protein that regulates the proteolytic activity of calpains (Schenkel et al. 2006) and according to Greenwood et al. (2013), the CAPN1-CAST marker profile is able to explain about 44% of the cooked beef tenderness variation. Another major contributor to meat tenderness is the IMCT, which contributes to the background toughness of meat. In skeletal muscle, based on its anatomical location, IMCT is divided into three different structures that are continuously connected: the endomysium, which enfolds each muscle fiber; the perimysium, which integrates all muscle fiber bundles; and the epimysium, which envelops the whole muscle (Nishimura 2010; Purslow 2014). The detailed structure and composition of IMCT can be found in previous reviews (McCormick 1994; Purslow 2002). The enzymes responsible for the degradation of IMCT are the matrix metalloproteinases (MMPs), a family of zinc-dependent proteases

(Woessner Jr 1991; Purslow 2014), and they are important targets for the improvement of meat tenderness through reducing of its background toughness.

1.2. Genetic influence on meat quality

1.2.1. Major gene effects

To put it simply, if a gene has a large effect on or can explain a large percentage of the variation of a certain trait, it is considered a major gene. But how large is considered large? According to Sellier and Monin (1994), if the difference in phenotypic value of a certain trait measured on the individuals carrying the homozygous genotype of a gene is larger than one standard deviation of the measured value compared with the ones that do not carry the same alleles of the gene, we consider that gene as a major gene influencing the trait. Major genes can be detected easily by just using the phenotypic data from different families (Sellier and Monin 1994; De Vries et al. 2000). Until now, there are a few major genes that have been detected that influence meat quality, including the halothane gene (*HAL*), the *Rendement Napole* (RN^-) gene, the *MSTN* gene, the *DGAT* gene, the *CAPN1* gene, and the *CAST* gene. Very good reviews can be found elsewhere for the *halothane* gene (Simpson and Webb 1989; Sellier and Monin 1994; Hermesch 1997; De Vries et al. 2000), the RN^- gene (Fernandez and Tornberg 1991; Ellis et al. 1999; De Vries et al. 2000; Rosenvold and Andersen 2003), the *MSTN* gene (Bellinge et al. 2005), the *DGAT* gene (Ma et al. 2005; Yen et al. 2008), the *CAPN1* and the *CAST* gene (Casas and Kehrli Jr. 2016), therefore, only a brief introduction is given here.

Halothane Gene

The *halothane* (*Hal* or *ryr*-1) gene, encoding the muscle ryanodine receptor (Fujii et al. 1991), is commonly referred to as the *porcine stress syndrome* (*PSS*) gene, and it has become known as such because swine that carried this gene exhibited symptoms associated with the

porcine stress syndrome when treated with halothane gas (Eikelenboom and Minkema 1974). Pigs that are homozygous (genotype *nn*) carriers of the *halothane* gene have limited ability to control calcium release into the sarcoplasm of the muscle cell due to a mutation in the ryanodine receptor/calcium release channel, and are susceptible to stress, with death being the most intense reaction (Cassens et al., 1975, Webb et al., 1982; Huff-Lonergan and Sosnicki 2002). In the pig production industry, it has been shown that *halothane* gene carriers produce more lean superior meat than their non-carrier counterparts (Oliver et al. 1993), with higher mortality rates and more PSE meat being harvested (Murray and Johnson 1998).

RN^{-} Gene

The *RN*$^{-}$ gene, named after the Rendement Napole (RN) test, is the major gene causing 'acid meat' (very low ultimate pH with rapid pH drop postmortem) (Naveau 1986; De Vries et al. 2000). In terms of meat quality, the *RN*$^{-}$ gene causes abnormal accumulation of glycogen in skeletal muscle, which increases its glycolytic potential and leads a dramatic decline of pH very early postmortem. As a result, muscle with low pH while it is still warm often develops PSE meat (Josell et al. 2000). A dominant mutation of the codon 200 of *PRKAG3* (protein kinase AMP-activated non-catalytic subunit gamma 3), mainly found in animals with a Hampshire background, was identified by Milan et al. (2000), which was responsible for the substitution of arginine by glutamine in the *RN*$^{-}$ gene (Milan et al. 2000) and, in 2001, three nonsynonymous substitutions (I199V, 199V-200R, 199I-200R) in the *PRKAG3* gene were detected by Ciobanu et al. (2001).

MSTN gene

In animal breeding, bovine muscular hypertrophy, commonly referred to 'double muscled', has become widespread among some Europen beef cattle breeds since 1888 (Bellinge et al. 2005). This phenomenon is caused by both an increase in the number of muscle fibres as well as an increase in myofibrillar protein accretion (Fiems 2012) and arises from the inhibition of myostatin activity, resulting in an exaggerated muscle development. *MSTN* (*Myostatin*), also known as *GDF8* (*growth differentiation factor 8*), is a protein coding gene and research has shown that variants of *MSTN* genes are associated with muscle hypertrophy and the gene is highly conserved across mammalian species (Grobet et al. 1997; McPherron et al. 1997; Mosher et al. 2007). By using a candidate approach, Grobet et al. (1997) found an 11-bp deletion in the coding sequence of the bioactive carboxy-terminal domain of the protein responsible for the muscular hypertrophy of Belgian Blue cattle. In another report from the same year, besides the 11-nucleotide deletion in the third exon of *MSTN* gene in Belgian Blue cattle, researchers found a missense mutation in exon 3 of *MSTN* gene, which caused a substitution of tyrosine by an invariant cysteine in the protein, resulting in double-muscled Piedmontese cattle (McPherron and Lee 1997). Although double muscled cattle have additional muscle disproportionately in the expensive cuts of meat (Bellinge et al. 2005), there are some serious defects within this phenotype, including reduced fertility, dystocia, low calf viability and increased disease susceptibility (Arthur et al. 1988; Arthur et al. 1989). This phenotype has persisted and in fact been purposefully selected because cattle with the *MSTN* gene have muscle with less intramuscular insoluble collagen and smaller muscle fiber cross-sectional area, leading to increased tenderness (Allais et al. 2010). The muscle also has reduced intramuscular fat and a less desirable flavour, but these factors are outweighed by the increased tenderness of the meat (Wiener et al. 2009).

DGAT gene

DGAT is a protein coding gene that encodes the microsomal enzyme Acyl CoA: diacylglycerol acyltransferase, which catalyzes the final step in triacylglycerol synthesis (Cases et al., 1998). There are two major types of DGAT, type 1 (DGAT1) and type 2 (DGAT2) that are encoded by *DGAT1* and *DGAT2*, respectively (Yen et al. 2008). Based on previous research, a lysine/alanine polymorphism in *DGAT1* gene has been shown to be involved in milk fat content (Grisart et al. 2002; Winter et al. 2002) and according to Thaller et al. (2003), the lysine allele of *DGAT1* may have a positive effect on intramuscular fat content. By investigating the candidate SNPs in the exon region of *DGAT1* gene in Chinese commercial cattle, two SNPs were found to be associated with back fat thickness, longissimus muscle area, marbling score, fat color and Warner-Bratzler shear force (Yuan et al. 2013).

CAPN1 and *CAST* gene

Two well-characterized calcium-dependent neutral proteinases in skeletal muscle are μ- and m-calpain, with μ-calpain mainly responsible for postmortem degradation of myofibrillar proteins and tenderization of meat (Geesink et al. 2000; Koohmaraie et al. 2002; Geesink et al. 2006). The gene code for μ-calpain is *CAPN1* (*Micromolar calcium activated neutral protease*) and this gene is located on bovine chromosome 29, and SNPs have been identified in this gene that influence meat tenderness (Page et al. 2002; Casas and Kehrli Jr. 2016). The natural inhibitor of both calpains (m- and μ-calpain) is calpastatin, which is coded by the *CAST* gene, which is located on bovine chromosome 7 (Casas and Kehrli Jr. 2016). According to Schenkel et al. (2006), a SNP in the *CAST* gene (a G to C substitution) was associated with beef tenderness. Combining *CAPN1* and *CAST*, the marker profile can explain about 44% of the variation in cooked beef tenderness (Greenwood et al. 2013).

1.2.2. Polygene effects

Like production and reproductive traits, it is also possible to improve meat quality using traditional selection methods (Larzul et al. 1997; Oksbjerg et al. 2004; Li et al. 2010). However, in practice, most meat quality traits are not easy to select for because of their low to moderate heritability, ranging from 0.15 to 0.30 in pork (Sellier and Monin 1994) and 0.10 to 0.30 in beef (Warner et al. 2010), and that they are difficult and expensive to measure. It is generally understood that meat quality traits are controlled by many genes because they are influenced by a multitude of factors (Gao et al. 2007). For such traits, marker-assisted or genomic selection has irreplaceable advantages compared with traditional selection due to its efficiency and reduced cost (Meuwissen and Goddard, 1996). One prerequisite for marker-assisted or genomic selection is the identification of quantitative trait loci (QTL) that correlate with variation in a phenotype and this has become possible now that the complete bovine and swine genomes have been assembled (Zimin et al. 2009; Elsik et al. 2009; Groenen et al. 2012). In recent years, whole genome scanning using dense SNP markers to identify QTLs affecting meat quality evolved to be a gold standard method in marker-assisted selection or genomic selection after the reference genome sequence was available. Many studies have been done related to pork quality (Luo et al. 2012; Ma et al. 2013; Ma et al. 2014; Xiong et al. 2015; Sato et al. 2016; Won et al. 2018) and beef quality (Gill et al. 2009; Zhou et al. 2010; Bolormaa et al. 2011; Li et al. 2012; Dunner et al. 2013; Lee et al. 2014; Srikanth et al. 2015; Magalhães et al. 2016; Santiago et al. 2017; Sant'Anna et al.) using SNP markers. However, none of those studies performed a comprehensive analysis on objective and subjective meat quality traits or considered if samples were fresh or frozen and then thawed or studied different species, and only a few of those studies combined meat science, biochemistry, genetics and functional genomics together to elucidate the relationships with meat quality.

1.3. Objectives of the thesis

As meat quality is becoming one of the most important factors contributing to the success of the meat industry, and improving meat quality attributes through animal breeding will benefit animal production, meat science, as well as human living standards, this thesis aimed to explore the possibility of improving important meat quality traits genetically in the future by addressing the following hypotheses: 1) There are variations (SNPs) in genes that are associated with dark-cutting beef; 2) Meat pH and intramuscular crude fat contribute differently to pork chop sensory acceptability. The genetic correlation between pH and pork sensory traits is different from that of intramuscular crude fat and sensory traits; 3) Genetic parameters estimated from meat quality data obtained from fresh pork will be different to those estimated from data on frozen-thawed pork; and 4) there are variations (SNPs) in genes along the genome that are associated with total collagen and collagen solubility. The direct objectives of this thesis were: 1) To identify SNPs along the genome that are associated with dark cutting beef and to explore the biological relevance of these SNPs to the formation of dark cutting beef; 2) To estimate the heritability for sensory traits, as well as their genetic and phenotypic correlations with meat pH and intramuscular fat content and to clarify the effect of pH and crude fat on the sensory traits of pork; 3) To estimate heritability, phenotypic, genetic and environmental correlations of meat quality measurements of fresh and frozen-thawed pork and to assess the effect of crude fat content on meat quality measurements performed on fresh and frozen-thawed pork; 4) To identify SNPs along the genome that are associated with bovine collagen solubility, and to explore the biological relevance of these SNPs to the toughness of meat. The long term objective was to genetically improve important meat quality traits to provide high quality meat for consumers in the future.

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2. A genome-wide case-control association study of dark cutting in beef cattle

2.1. Introduction

Dark cutting beef, also known as dark, firm and dry (DFD) beef, is beef with an abnormal dark red colour when compared to that with a normal bright red colour after exposure to oxygen. Because of its visually unappealing dark colour, dark cutting beef may be perceived as tough beef from old animals and thus discriminated against by consumers. In addition, because of its relatively high ultimate pH values (pH > 5.8), dark cutting beef is an ideal supporter for the growth of spoilage bacteria, leading to the reduction of its shelf-life (Tarrant 1989). With these defects, the occurrence of dark cutting beef will cause tremendous damage to the quality value of a carcass, resulting in non-negligible economic loss for beef producers and the beef industry. According to the National Beef Quality Audit (NBQA) report in 1995, the occurrence rate of dark cutters was 2.7% (Boleman et al. 1998), resulting in a loss of about $6 per head in the United States (Scanga et al. 1998). In Canada, the incidence of dark cutters (Canada B4) found in youthful cattle has increased from 0.84% in 1998/1999 to 1.28% in 2010/2011 (Beef Cattle Research Council (BCRC), 2013). In Australia, the dark cutting rate was about 5.9% in the 2014-2015 financial year (Loudon et al. 2018).

In beef production, muscle postmortem anaerobic glycolysis, using glycogen as its substrate, reduces intracellular muscle pH from the physiological value of around 7.0 (Tarrant 1989) to 5.7 or lower after the animal is slaughtered, allowing the normal aging of meat to proceed. One result of the normal aging of meat is colour development following the decline of pH: with the decline of intramuscular pH after slaughter, muscle mitochondrial oxygen consumption will be compromised, leading to the accumulation of oxy-myoglobin formed from the oxygenation of myoglobin, resulting in the appearance of the bright red colour with exposure to atmospheric oxygen (Egbert and Cornforth 1986). However, if animals experience chronic or

long term physical and/or psychological stress before slaughter, depletion of muscle glycogen may occur, leading to inadequate anaerobic glycolysis postmortem. With low intramuscular glycogen concentration, below 66 μmol glucose g^{-1} of muscle (Hansone et al. 2001), or depletion of muscle glycogen before slaughter, anaerobic glycolysis of muscle will not proceed, leaving the muscle pH value higher than 5.8 and causing the reduction of meat shelf-life, as well as the appearance of an undesirable dark colour. Examples of pre-slaughter stressors include the change of adapted environment (noise, odours, temperature, humidity, dwelling environment), breakdown of social groupings and hierarchies, improper loading and/or unloading during transportation, confinement or overcrowding, and the deprivation of food and water (Warriss 1990).

There is consensus on the causes of dark cutting beef; specifically, that pre-slaughter chronic or long term environmental stress or acute or short term stress can lead to the occurrence of dark cutting (Adzitey and Nurul 2011). However, it is obvious that there is variation in the response to environmental stress among individuals or breeds. Although tropical breeds have been cited as being better than temperate breeds in terms of stress resistance (Adzitey and Nurul 2011), Muchenje et al. (2009) found that Bonsmara steers showed greater acute stress responsiveness when exposed to similar pre-slaughter handling conditions compared to cattle from the Angus and Nguni breeds (Muchenje et al. 2009). In terms of thermoregulation and grazing, the Bonsmara-Hereford crossbred showed better performance than the Hereford in high temperature conditions (Taborda et al. 2018). With these results in mind, we hypothesized that genetic variation may be contributing to variation in the occurrence of dark cutting beef and that there is a genetic effect on the incidence of dark cutting beef. Analysis for genetic effects on dark cutting is complicated by dark cutting not being a quantitative trait; the LT muscle is either dark

or it is not. Although there is the possibility of using intramuscular pH as a quantitative measure of dark cutting as dark cutting beef usually has a pH value greater than 5.8, intramuscular pH is not an absolute indicator of dark cutters because some dark cutters have pH values lower than 5.8 (Mahmood et al. 2017). There is also no continuous objective colour distribution available for dark cutters using an objective colour space such as the Commission International de l'Eclairage (CIE) L*a*b*, and so it is not possible to analyze dark cutting beef as a quantitative trait.

When a dataset has two primary categories of phenotype (normal, dark), it lends itself to analysis as a binary case/control study (Bush and Moore 2012). Case control GWAS are particularly applicable when the phenotype is clearly classified as either affected or unaffected by the condition of interest, and when the assessment of whether an organism is affected or unaffected is governed by standardized descriptions of both phenotypes that are applied reproducibly (Bush and Moore 2012). In the case of dark cutting, personnel trained by the Canadian Beef Grading Agency are trained to reproducibly ascertain dark cutting carcasses by inspecting the 12^{th}-13^{th} rib m. *longissimus thoracis* interface for its redness against a plastic colour chart, thus meeting this criterion. Case-control GWAS has recently been used to identify SNPs associated with lung lesions and liver abscesses from beef cattle samples collected at slaughter using a pooling approach (Keele et al. 2015; 2016).

Examination of genes that may contribute to the occurrence of dark cutters is further complicated by the difficulty in assembling large data sets that include a sufficient number of dark cutting cattle with genotypes. This difficulty arises due to the sporadic and low level of incidence of dark cutting in the slaughter cattle population, making continuous sampling for genetic analysis unpredictable and thus uneconomical. As a result, databases that do contain dark

cutting cattle are often small, collected over many years, and have a disproportionate number of normal to dark cutting carcasses, thus prohibiting definitive genome wide associative studies. Although case-control GWAS studies have less power than quantitative trait GWAS (Van der Sluis et al. 2013), case-control GWAS offers the opportunity to match "cases" (dark cutters) with controls selected specifically to match phenotype and/or environment (e.g. slaughter batch) to minimize variation between cases and controls and strengthen the analysis (Bush and Moore 2012).

Hence, a case-control GWAS was performed with the aim to: 1) to identify variations in genes associated with dark cutting beef; and 2) to explore the biological relevance of the genes in the formation of dark cutting beef.

2.2. Materials and methods

All animals used in this study were managed according to the guidelines of Canadian Council on Animal Care (CCAC 1993) and the experimental procedures were approved by an ethics committee at the University of Alberta (AUP00000777).

2.2.1. Animal management and phenotype data

The animals used in this study came from two beef cattle groups, where Group I had 64 beef cattle of which 40 were graded Canada B4 (dark cutters, treated as cases) and detailed information of how these samples were abtained can be found elsewhere (Mahmood et al. 2017), and Group II had 837 beef cattle in total, of which 30 were graded Canada B4 (Table 2.1). This was reduced to 150 (120 controls and 30 cases) for statistical reasons (see below). For Group I, there was no detailed breed information, and so breed composition was predicted for all individuals using a cross-validation procedure implemented in ADMIXTURE software (Alexander et al. 2009) using 50k genotypes. The cross-validation procedure was performed 5-fold in ADMIXTURE for K values from 1 to 20. To define each of the ancestries (i.e. k, the

number of populations), the breed proportions produced by ADMIXTURE were aligned with breed information from an existing pedigree having individuals who are strongly assigned to particular breed or population. Detailed information about the reference population can be found elsewhere (Abo-Ismail et al. 2016). This analysis indicated that all 64 beef cattle were crossbred with Angus (0.01-0.92), Hereford (0.02-0.94), Simmental (0.00-0.76), Charolais (0.00-0.81), Limousin (0.00-0.47) and Gelbvieh (0.00-0.43) genetics. The beef rib samples (m. *longissimus thoracis*; n = 64) from these heifers (12 controls and 23 dark cutters) and steers (12 controls and 17 dark-cutters) were collected from a commercial beef abattoir 24 - 48 h post-mortem after carcasses were graded by trained personnel from the Canada Beef Grading Agency. Carcasses with colour intensity above the federally-approved borderline were graded Canada B4, omitting further assessment for marbling, subcutaneous fat, or muscling. For Group II, in total, 837 carcasses (377 steers, 460 heifers) from four crossbred lines: CHAR (Charolais × Red Angus, n = 97), HEAN (Hereford × Angus, n = 81), HEANGV (Hereford-Angus × Gelbvieh-Angus, n = 147) and TXX (produced from crosses between a composite terminal bull strain which was derived from Hereford, Black Angus, Red Angus, and Limousin, and crossbred cows with a mixed background of Angus, Red Angus, Hereford, Simmental, Charolais, Limousin and Gelbvieh, n = 512), were graded by trained personnel from the Canada Beef Grading Agency using the same procedure as Group I, resulting in 30 dark cutters and 807 normal carcasses. Based on previous research (Smith 1997; Austin 2010; Abadie and Imbens 2011; Rassen et al. 2012; Linden and Samuels 2013) using case-control study methods, to gain the most statistical power with the lowest bias in treatment effect, the number of controls may be up to four times the number of cases; that is, the control and case ratio can be as high as 4:1. In the present study, the number of controls far exceeded that of cases in Group II (807 controls and 30 cases). In

order to minimize treatment effect bias and maximize our statistical power using available resources, 120 controls were selected to achieve the 4:1 ratio in Group II by selecting animals physically similar (based on cattle breed and contemporary group information) to cases from 807 beef cattle, resulting in 150 individuals (30 cases and 120 controls) for association analysis for Group II. As for the Combined Groups, 136 additional control beef cattle were added from Group II (837) to match the 70 cases (40 in Group I and 30 in Group II), resulting in 280 controls and 70 cases (Table 2.1).

2.2.2. Quality control and population stratification

Group I individuals were genotyped using GeneSeek Genomic Profiler for Beef Cattle-HD (GGP-HD), which in total features 76783 SNPs (70k). Group II animals were genotyped using Illumina BovineSNP50v2 BeadChip, which in total features 54609 SNPs (50k). All SNPs with a missing rate higher than 10% or a minor allele frequency lower than 5% were removed. Before association studies, the top two principal components (PCs) were generated using all the genotype data through GCTA (Genome-wide Complex Trait Analysis) and were evaluated using R/ ggplot2.

2.2.3. Genome-wide association study

Genome-wide association analyses were conducted using Plink 1.9 (Chang et al. 2015). Dark-cutting beef was analyzed as a binary trait (cases versus controls) using a logistic regression model under an additive model. Possible confounding factors, like gender, batch, lot, lairage, slaughter date and slaughter weight for Group I; gender, breed, farm, contemporary group, slaughter date and slaughter weight for Group II, were tested using the glm function in R (R Core Team, 2013) and the significant factors (lairage for Group I, contemporary group and slaughter weight for Group II), as well as the first two PCs (the first two PCs already could capture population structure, as shown in Figure 2.1 (A, B)), were included in the logistic

regression model. Using multiple significance level criteria, the association results producing a nominal *p* value smaller than 0.01, 0.001 and 0.0001, respectively, and were reported as significant in this study. In addition, the nominal *p*-values were adjusted through the false discovery rate (FDR) (Benjamini and Hochberg 1995) using the R package R/stats, in which the FDR was obtained. An FDR less than 0.05 was considered statistical significant.

2.2.4. Functional analysis

The top 50 SNPs with the lowest nominal *p* values in Group I, Group II and the Combined Groups were reported in this study, and their nearest (within 1 Mb window) RefSeq genes were searched through Ensembl BioMart (Zerbino et al. 2017) (release 92) and used for the analysis of metabolic pathways, molecular and cellular functions, as well as gene networks using IPA, a web-delivered application used to identify, illustrate and investigate biological mechanisms, pathways and functional genes (http://www.ingenuity.com). The "Core Analysis' function included in IPA was used to interpret the data and the probability of each assigned gene function being due to chance alone was tested by the Fisher Exact test with a significance threshold of $p < 0.05$.

2.3. Results

2.3.1. Quality control and population structure

After quality control, there were 69474, 38801 and 23421 autosomal SNPs remaining for Group I, Group II and Combined Groups, respectively, and these were used in the genome-wide association analysis. The 23421 SNPs are common SNPs when Group I and Group II were combined. The plot (using top two PCs) generated by R comparing different breeds clearly showed clustering in Group I (Figure 2.1 A) and Group II (Figure 2.1 B), indicating the existence of population stratification. To adjust for this population stratification, the first two PCs were added as covariates in the logistic regression model while doing the association analysis.

2.3.2. Genome-wide association study

Using multiple statistical significance thresholds (p = 0.01, 0.001 and 0.0001), genome-wide association study through logistic regression under an additive model identified 449 SNPs in Group I, 301 SNPs in Group II and 209 SNPs in the Combined Groups with a significance level of $p < 0.01$; 12 SNPs in Group I, 21 SNPs in Group II and 18 SNPs in the Combined Groups with a significance level of $p < 0.001$; 3 SNPs in Group II and 1 SNPs in the Combined Groups with a significance level of $p < 0.0001$ (Table 2.2, Figure 2.2). However, none of these detected SNPs passed multiple test adjustment using an FDR threshold of 0.05 (Table 2.2). The top 50 SNPs with the lowest nominal p values in each group are shown in Tables 2.3 to 2.5.

2.3.3. Functional analysis

In total, 708 RefSeq genes were found harboring the 150 SNPs of all Groups with the lowest p values, and the molecules were involved in important functions like molecular transport, small molecule biochemistry and carbohydrate metabolism. For molecular and cellular functions, in total, 21 functions were generated using the threshold of $p < 0.05$ (Figure 2.3), among which carbohydrate metabolism was a function of interest as it may be related to the formation of dark cutting beef.

Under the function of carbohydrate metabolism, 64 genes were involved in important biological processes, including conversion of D-glucose (4 genes), quantity of phosphatidylethanolamine (5 genes), quantity of carbohydrate (38 genes), uptake of 2-dexoyglucose (11 genes), synthesis of carbohydrate (30 genes) and metabolism of carbohydrate (41 genes) (Table 2.6). Because glycogen is responsible for the formation of dark cutting beef, the detailed functions of the genes related to carbohydrate metabolism were worthy of further examination (Figure 2.4).

2.4. Discussion

The occurrence of dark cutters is difficult to predict, thus contributing to the difficulty in collecting case samples for research investigating the incidence of dark cutting beef. Considering the sample size we used and the incidence of dark cutting, it is reasonable to report results with less stringent statistical significance level. Normally, for genome-wide association study, thousands of experimental individuals should be used to gain reasonably acceptable statistical power to detect possible associations, both for case-control and quantitative traits (Spencer et al. 2009; Hong and Park 2012; Visscher et al. 2012). For genome-wide case-control study, a large sample size is needed if the prevalence of a trait of interest is low, so as to gain the same statistical power as a study where the traits of interest were of high prevalence (Hong and Park 2012). In the present study, with the low incidence of dark cutting, a large sample size is needed to detect possible SNPs associated with the phenotype. In our analyses, however, only 64, 150 and 350 beef cattle were used for association analysis in Group I, Group II and a Combined Group, respectively. With the increased sample size for the Combined Group (n = 350) compared with Group I (n = 64) and Group II (n = 150), only one SNP passed the $p < 0.0001$ threshold (SNP ARS-BFGL-NGS-21302 from Group II with a nominal p value of 0.00003912), which might indicate that the sample size of the Combined Group was still insufficient, and that different genotyping panels were used for Group I and Group II most likely contributed to this insufficiency, as it resulted in only 27642 common SNPs after combining the two panels together. Based on the results, we can also speculate that the formation of dark cutting beef may be controlled by many genes with a small effect of each; that is, the trait may be polygenic as there was no evidence of major effect loci. Although no SNPs passed the FDR test, there were SNPs that did show trends of association with dark cutting. To provide basic information for future genetic research related to dark cutting beef, the 50 associated SNPs throughout the genome with

lowest *p* values in each Group (Group I, Group II and Combined Group) were reported and used for further exploration of the biological meaning of the study.

The intuitive way of exploring the biological relevance of the RefSeq genes near the top 50 SNPs in each Group is to obtain common genes found in Group I, Group II and the Combined Group. Unfortunately, there were only 4 common genes obtained (Figure 2.5), and they were not related to carbohydrate metabolism, which might be responsible for the formation of dark cutting beef post-mortem. Why there were so few common genes is most likely due to different genotyping panels being used for Group I and Group II, resulting in fewer common SNPs for the Combined Group analysis. The alternate way to address this problem then was to treat each Group separately and use all the RefSeq genes harboring the 50 SNPs with the lowest *p* values generated from Group I, Group II and Combined Group, respectively, which is what we did in this study. Another way is to impute the missing genotypes. Using Fimpute v2.2 (Sargolzaei et al. 2014), Group II panel was imputed to group I panel (from 50k to 70k). However, among the top 50 SNPs in Group I and Group II, there were no overlapping SNPs, which means, these 50 SNPs in Group II were lost after imputation. Plus, the association using imputed dataset did not detect significant SNPs (FDR < 0.05) (Supplementary Table 2.1), so these results were not further discussed.

The eating qualities of meat can be developed through postmortem muscle aging, during which meat tenderness will be improved dramatically (Pierson and Fox 1976). The basic physical changes underlying the conversion of muscle to meat is driven by the switch from the dominant aerobic bio-energetic system in live animals to the postmortem anaerobic glycolysis system which uses glycogen as its main substrate after about 70% of the phosphocreatine (PCr) pool has been degraded (Bendall 1951). This leads to the proper acidification of meat caused by

hydrogen ions generated through anaerobic glycolysis. Anything related to this process may be responsible for the high ultimate pH (Page et al. 2001) of dark cutting beef, and glycogen is used as the substrate for postmortem muscle anaerobic glycolysis, which accumulates hydrogen ions, leading to the proper aging of meat. Hence, the quantity of glycogen and/or the rate of glycogen metabolism postmortem are therefore worthy of attention. From the IPA results, 60 genes were found related to the quantity, synthesis, and metabolism carbohydrate, and included genes such as *DGAT2* (*diacylglycerol O-acyltransferase 2*), *FOXO1* (*forkhead box O1*), *GHR* (*growth hormone receptor*), *P2RY2* (*purinergic receptor P2Y2*), *PGM2* (*phosphoglucomutase 2*), and *PRKAB2* (*protein kinase AMP-activated non-catalytic subunit beta 2*). IPA is a pathway-based tool which uses its own respective curated pathway collections (Cirillo et al. 2017), but does not include genes for cattle. As the functional analysis of the GWAS-region RefSeq genes described in this thesis was performed using IPA, there may be some inconsistencies regarding gene function due to differences between the species, as genes included in IPA are from human, mouse, and rat. From IPA, the most representative processes were obtained, including canonical pathways, networks, upstream regulators, diseases and biological functions. Important genes or gene functions could have been missed due to differences between the species or due to lack of understanding of all the functions of the genes identified by IPA. Conversely, some of the reported processes may not actually be relevant to the traits of interest but instead could arise through imperfect gene annotations and imprecise GWAS region boundaries. Based on these limitations, the candidate genes discussed in this thesis should be interpreted with caution and the relationship of the genes with dark cutting beef validated before being used for selection purposes.

DGAT2 plays important roles in triglyceride synthesis (Cases et al. 2001) and intra-myocellular triglyceride synthesis could minimize the formation of glycogen due to its role in ensuring the proper endurance of physical activity (Jonhson et al. 2004). This is why cattle with different levels of fatness may have different levels of glycogen concentration post-mortem, which derives the possible correlation of intramuscular fat level and the formation of dark cutting meat worthy of note in the future. Forkhead box O1, coded by the *FOXO1* gene, can significantly decrease the incorporation of glucose into glycogen with a modest effect (Bastie et al. 2005), and hence, may negatively influence the synthesis of glycogen in skeletal muscle. According to Allen and Unterman (2007), *FOXO1* transcription factors play a role in increasing the expression of myostatin (Allen and Unterman, 2007) and therefore, may suppress skeletal muscle growth.

Based on the IPA results, the *GHR* (*growth hormone receptor*) gene was involved in determining the quantity of carbohydrate and the *P2RY2* (*purinergic receptor P2Y2*) gene was involved in determining the quantity of carbohydrate, the synthesis of carbohydrate and the metabolism of carbohydrate. According to Sibut et al. (2011), *GHR* was overexpressed in chicken muscles with high glycogen content and *P2RY2* was overexpressed in muscles with low glycogen content. This result needs to be further validated to confirm if *P2RY2* and *GHR* play roles in muscle glycogen metabolism. *Phosphoglucomutase 2* (*PGM2*) is a protein coding gene, responsible for the glucose metabolism pathway. Phosphoglucomutase catalyzes the interconversion of glucose-1-phosphate and glucose-6-phosphate (Quick et al. 1972), which is an essential intermediate step of glycogen synthesis. In mouse tissues, the PGM2 isozyme contributed most of the phosphoglucomutase activity relative to the isozymes PGM1 and PGM3. However, no study has been performed in beef cattle to measure the contribution of PGM2 to the

overall phosphoglucomutase activity. Protein kinase AMP-activated non-catalytic subunit beta 2 isoform, coded by *PRKAB2*, is highly expressed in skeletal muscle compared to its counterpart beta 1 isoform, and is a subunit of AMP-activated protein kinase (AMPK) (Thornton et al. 1998). AMPK plays an important role in calpastatin gene transcription through the stimulating of calcium transportation (Mickelson et al. 1985; Cong et al. 1998; da Silva et al. 2016), and hence, may be influencing meat tenderness. Also, protein kinase AMP-activated non-catalytic subunit beta 1/2, with a glycogen binding domain, may regulate AMPK interaction with glycogen particles and ultimately regulate metabolic energy balance (Hardie et al. 2012).

Besides all these genes, gene *CHD4* (*Cadherin 4*) is worthy of further discussion because the most significant SNP detected in Group II (SNP name: ARS-BFGL-NGS-21302, Chromosome: 13, Position: 55973589, *p* value: 0.00003912) was located in the intron of this gene, suggesting a possible role in the formation of dark cutting beef. According to Welle et al. (2009), *CHD4* gene expression was upregulated two fold in the skeletal muscle of myostatin knockout mice, indicating its possible role in regulating muscle development. Cadherin 4 (also known as R-cadherin), encoded by *CHD4* gene, is a calcium-dependent cell-cell adhesion glycoprotein serving as a central morphogenetic regulator (Inuzuka et al. 1991; Takeichi, 1995; Rossenberg et al. 1997). R-cadherin is a well-known classic cadherin identified in chicken (Inuzuka et al. 1991), mouse (Hutton et al. 1993; Matsunami et al. 1993) rat, and human (Suzuki et al. 1991). After examining the embryonic expression pattern of R-cadherin in mouse, Rossenberg et al. (1997) detected very high levels of expression of R-cadherin in early skeletal muscle, suggesting a possible role of R-cadherin during secondary myogenesis. Comprehensive research conducted by Kucharczak et al. (2008) revealed that R-cadherin expression in C2C12 myoblasts caused inhibition of myogenesis induction, diminishing its importance as a causal

agent for muscle precursor cell proliferation and/or migration during embryonic development (Kucharczak et al. 2008).

The discussion above revealed that the 50 most significant SNPs identified in the three Groups may play important roles in glycogen turnover in skeletal muscle, however, further validation is needed to ensure if they are truly associated with the formation of dark cutting beef. Future research should focus on the detection of SNPs associated with dark cutting beef in a much larger group of beef cattle than currently analyzed, and/or the validation of the 50 most significant SNPs identified in this study in a different beef cattle population, and/or the validation of the detected RefSeq genes via q-PCR. Although no profoundly significant SNPs were found in the present study, the results did show some association between genetic variants and the formation of dark cutting beef in the detailed analysis of the biological relevance.

2.5. Conclusions

Based on our association study with a relatively small sample size, no strong evidence was found for a large influence of any one gene in the incidence of dark cutting beef, suggesting that the trait may be polygenic. However, based on the functional analysis, the most significant SNPs did show suggestive association with dark-cutting beef as the nearest genes had biological relevance to the formation of dark cutting. Although the detected SNP associations require validation in a dataset much larger than that studied in this work, the results suggested the possibility in the future for marker-assisted selection or genomic selection in beef cattle to reduce dark cutting.

Table 2.1. Number of beef cattle been used in each Group.

Animal Groups	Number of individuals		
	Case	Control	Total
Group I	40	24	64
Group II	30	120	150
Combined Groups*	70	280	350

[1] The Combined Groups is not just the sum of Group I and II, more control animals were selected from the total Group II to meet the 4:1 ratio standard of control:case.

Table 2.2. Number of significant SNP at different statistical significance thresholds ($p < 0.01$, $p < 0.001$ and $p < 0.0001$) for all beef cattle groups studied.

	Number of SNPs			
Groups	$p < 0.0001$	$p < 0.001$	$p < 0.01$	HB_FDR < 0.05
Group I	0	12	449	0
Group II	3	21	301	0
Combined Groups	1	20	191	0

Table 2.3. Fifty (50) associated SNPs with the lowest *p* values in Group I.

Ranking	SNP ID	Chromosome	Position	*p*-value	HB_FDR
1	BTB-00259302	6	61640981	0.0003973	0.894953
2	BovineHD2200000640	22	2495848	0.0004284	0.894953
3	BTB-00207198	21	44574752	0.0005	0.894953
4	ARS-BFGL-NGS-111563	11	85740344	0.0005488	0.894953
5	BovineHD0300015622	3	51650834	0.0005713	0.894953
6	BovineHD1300004299	13	15171277	0.0006534	0.894953
7	BovineHD0800003717	8	11346662	0.0006722	0.894953
8	BovineHD0900016028	9	58600581	0.0007841	0.894953
9	Hapmap58422-rs29021136	15	55961906	0.0008038	0.894953
10	ARS-BFGL-NGS-40602	15	53250782	0.0008332	0.894953
11	BovineHD2700009653	27	34229057	0.0009115	0.894953
12	BovineHD1400018736	14	67010904	0.0009831	0.894953
13	BTB-00079551	2	9454144	0.001051	0.894953
14	BovineHD0500015579	5	54634733	0.001053	0.894953
15	Hapmap39304-BTA-109342	11	85779114	0.001102	0.894953
16	BovineHD0200021281	2	74246725	0.001132	0.894953
17	BovineHD1000017602	10	60394494	0.001182	0.894953
18	BovineHD0600017007	6	61675649	0.001221	0.894953
19	BovineHD1300004322	13	15250019	0.001228	0.894953
20	Hapmap36462-SCAFFOLD15048_5152	18	50581375	0.001278	0.894953

21	BTA-34161-no-rs	13	15146915	0.00134	0.894953
22	ARS-BFGL-NGS-113489	13	15224026	0.00134	0.894953
23	ARS-BFGL-NGS-104166	2	74548518	0.001355	0.894953
24	BovineHD1900013865	19	49685493	0.001392	0.894953
25	BovineHD2700009737	27	34605717	0.001414	0.894953
26	BTB-01174913	23	48407046	0.001417	0.894953
27	BovineHD1300004307	13	15196458	0.001473	0.894953
28	BovineHD0800007319	8	24348870	0.001485	0.894953
29	BovineHD2400010634	24	38882678	0.001563	0.894953
30	BovineHD2400010619	24	38800620	0.001574	0.894953
31	BovineHD1800000982	18	3402621	0.001632	0.894953
32	BovineHD1100002429	11	6488794	0.00164	0.894953
33	ARS-BFGL-NGS-108861	27	34758932	0.001707	0.894953
34	BovineHD0300015191	3	49863995	0.00172	0.894953
35	Hapmap55817-rs29016350	10	83762976	0.0019	0.894953
36	BovineHD0600007965	6	28722959	0.001937	0.894953
37	ARS-BFGL-BAC-28969	2	30197027	0.002019	0.894953
38	BovineHD0800021430	8	71272353	0.00203	0.894953
39	BovineHD1800001013	18	3554476	0.002055	0.894953
40	BovineHD1200006478	12	21509307	0.002119	0.894953
41	BovineHD0300007153	3	22852111	0.002138	0.894953
42	Hapmap25231-BTA-120294	2	70609758	0.002149	0.894953
43	BovineHD2200010545	22	37102808	0.002235	0.894953

44	BovineHD1600010245	16	35655692	0.002323	0.894953
45	BovineHD1900013935	19	49950351	0.002346	0.894953
46	BovineHD0800021534	8	71518617	0.002427	0.894953
47	UA-IFASA-7069	20	31933394	0.002431	0.894953
48	BovineHD0200024861	2	87403759	0.00246	0.894953
49	BovineHD2500005213	25	18544524	0.002463	0.894953
50	BovineHD0400011751	4	42916786	0.002499	0.894953

Table 2.4. Fifty (50) associated SNPs with lowest *p* values in Group II.

Ranking	SNP ID	Chromosome	Position	*p*-value	HB_FDR
1	ARS-BFGL-NGS-21302	13	55973589	0.00003912	0.957326
2	ARS-BFGL-NGS-103191	3	109702946	0.00006842	0.957326
3	BTB-01125910	13	51067877	0.00009398	0.957326
4	BTB-01157295	8	43919283	0.0001165	0.957326
5	BTB-01125985	13	51118474	0.0002117	0.957326
6	BTA-98940-no-rs	2	77832115	0.0002225	0.957326
7	ARS-BFGL-NGS-27002	5	79357868	0.0002426	0.957326
8	UA-IFASA-7071	19	23628220	0.0002866	0.957326
9	ARS-BFGL-NGS-42736	3	33367402	0.0002918	0.957326
10	ARS-BFGL-NGS-26624	3	117985787	0.0003728	0.957326
11	ARS-BFGL-NGS-101411	2	72163562	0.0003734	0.957326
12	BTB-00388242	9	34426533	0.0004074	0.957326
13	Hapmap43671-BTA-74719	5	98107528	0.0004136	0.957326
14	Hapmap46736-BTA-94583	16	10740538	0.0004407	0.957326
15	UA-IFASA-5538	6	58871346	0.0004865	0.957326
16	Hapmap25127-BTA-83222	9	34658951	0.0005438	0.957326
17	BTB-00244579	6	13502824	0.0006439	0.957326
18	BTA-102943-no-rs	2	78166743	0.000706	0.957326
19	ARS-BFGL-BAC-13049	11	64019136	0.0008114	0.957326
20	ARS-BFGL-NGS-39984	17	72414626	0.0008867	0.957326
21	ARS-BFGL-NGS-118771	3	34624789	0.0009798	0.957326

	ARS-USMARC-Parent-				
22	EF034081-rs29009668	13	25606469	0.001068	0.957326
	Hapmap36096-				
23	SCAFFOLD140080_30362	13	25606469	0.001068	0.957326
24	BTB-01241144	1	151615270	0.001106	0.957326
25	ARS-BFGL-NGS-72188	6	41831446	0.001116	0.957326
26	BTB-01817097	3	71038159	0.001153	0.957326
27	BTB-00388235	9	34453477	0.001273	0.957326
	Hapmap36338-				
28	SCAFFOLD140080_5028	13	25631340	0.00128	0.957326
29	Hapmap43745-BTA-101961	2	55341069	0.001333	0.957326
30	ARS-BFGL-NGS-65062	6	1.18E+08	0.001386	0.957326
31	Hapmap46780-BTA-18414	9	17864398	0.001413	0.957326
32	BTA-83295-no-rs	9	35083835	0.001423	0.957326
33	BTB-00567566	14	46959846	0.001459	0.957326
34	ARS-BFGL-NGS-19663	26	43933332	0.001503	0.957326
35	ARS-BFGL-NGS-84686	7	64451610	0.001519	0.957326
36	BTA-121196-no-rs	19	59441263	0.00152	0.957326
37	BTB-00389124	9	35036949	0.001526	0.957326
38	BTA-109914-no-rs	2	547782	0.001536	0.957326
39	ARS-BFGL-NGS-18439	12	88759054	0.001541	0.957326
40	Hapmap57941-rs29016512	26	18219181	0.001568	0.957326
41	Hapmap27597-BTA-146334	9	34199814	0.00161	0.957326

42	BTB-00436535	10	84388256	0.00161	0.957326
43	ARS-BFGL-NGS-84905	1	37209389	0.001626	0.957326
44	ARS-BFGL-NGS-11787	5	78714231	0.001654	0.957326
45	ARS-BFGL-NGS-14064	17	70814867	0.001703	0.957326
46	ARS-BFGL-NGS-1405	14	62508173	0.001765	0.957326
47	ARS-BFGL-NGS-52020	11	36808946	0.001915	0.957326
48	ARS-BFGL-NGS-116713	19	55024753	0.00193	0.957326
49	BTB-00635702	16	34994367	0.001962	0.957326
50	Hapmap40748-BTA-47979	2	70322692	0.001968	0.957326

Table 2.5. Fifty (50) associated SNPs with lowest *p* values in Combined Group.

Ranking	SNP ID	Chromosome	Position	*p*-value	HB_FDR
1	ARS-BFGL-NGS-21302	13	55973589	0.00003508	0.43059244
2	BTB-01125910	13	51067877	0.00004896	0.43059244
3	ARS-BFGL-NGS-103191	3	109702946	0.00005532	0.43059244
4	ARS-BFGL-NGS-101411	2	72163562	0.0001475	0.73415544
5	UA-IFASA-7071	19	23628220	0.0001572	0.73415544
6	UA-IFASA-5538	6	58871346	0.0003926	0.930329285
7	ARS-BFGL-NGS-39984	17	72414626	0.000395	0.930329285
8	Hapmap25127-BTA-83222	9	34658951	0.0004292	0.930329285
9	BTB-00388242	9	34426533	0.0004335	0.930329285
10	Hapmap46736-BTA-94583	16	10740538	0.000503	0.930329285
11	ARS-BFGL-NGS-42736	3	33367402	0.0005074	0.930329285
12	ARS-BFGL-NGS-27002	5	79357868	0.0006249	0.930329285
13	BTB-00244579	6	13502824	0.0006915	0.930329285
14	ARS-BFGL-NGS-26624	3	117985787	0.0007222	0.930329285
15	BTB-00502017	12	67951346	0.0008879	0.930329285
16	BTB-00567566	14	46959846	0.0009425	0.930329285
17	ARS-USMARC-Parent-EF034081-rs29009668	13	25606469	0.0009584	0.930329285
18	BTB-00436535	10	84388256	0.0009889	0.930329285
19	ARS-BFGL-NGS-39532	8	10623685	0.001184	0.930329285
20	Hapmap35135-	22	23765329	0.001277	0.930329285

	BES10_Contig779_1471				
21	BTB-00934994	29	23935266	0.001397	0.930329285
22	BTA-114272-no-rs	2	114133415	0.001428	0.930329285
23	ARS-BFGL-NGS-18439	12	88759054	0.001493	0.930329285
24	BTA-83295-no-rs	9	35083835	0.001504	0.930329285
25	ARS-BFGL-NGS-115705	9	19040720	0.001551	0.930329285
26	BTB-01934112	4	34059453	0.001575	0.930329285
27	ARS-BFGL-NGS-104344	8	37136374	0.001615	0.930329285
28	BTA-121196-no-rs	19	59441263	0.001906	0.930329285
29	Hapmap30912-BTA-71048	4	68313751	0.001953	0.930329285
30	ARS-BFGL-NGS-84686	7	64451610	0.001967	0.930329285
31	Hapmap33275-BTA-98050	11	52045971	0.001983	0.930329285
32	ARS-BFGL-BAC-13049	11	64019136	0.002114	0.930329285
33	BTB-00581462	15	13202638	0.002201	0.930329285
34	BTB-00389124	9	35036949	0.002245	0.930329285
35	BTB-01108785	4	21118823	0.002262	0.930329285
36	Hapmap40748-BTA-47979	2	70322692	0.002265	0.930329285
37	ARS-BFGL-NGS-19602	14	21286894	0.002319	0.930329285
38	BTB-01549056	8	64438267	0.002353	0.930329285
39	ARS-BFGL-NGS-117528	12	54550449	0.002374	0.930329285
40	Hapmap27597-BTA-146334	9	34199814	0.002376	0.930329285
41	BTB-00753516	11	19344832	0.00238	0.930329285
42	ARS-BFGL-NGS-116713	19	55024753	0.002394	0.930329285

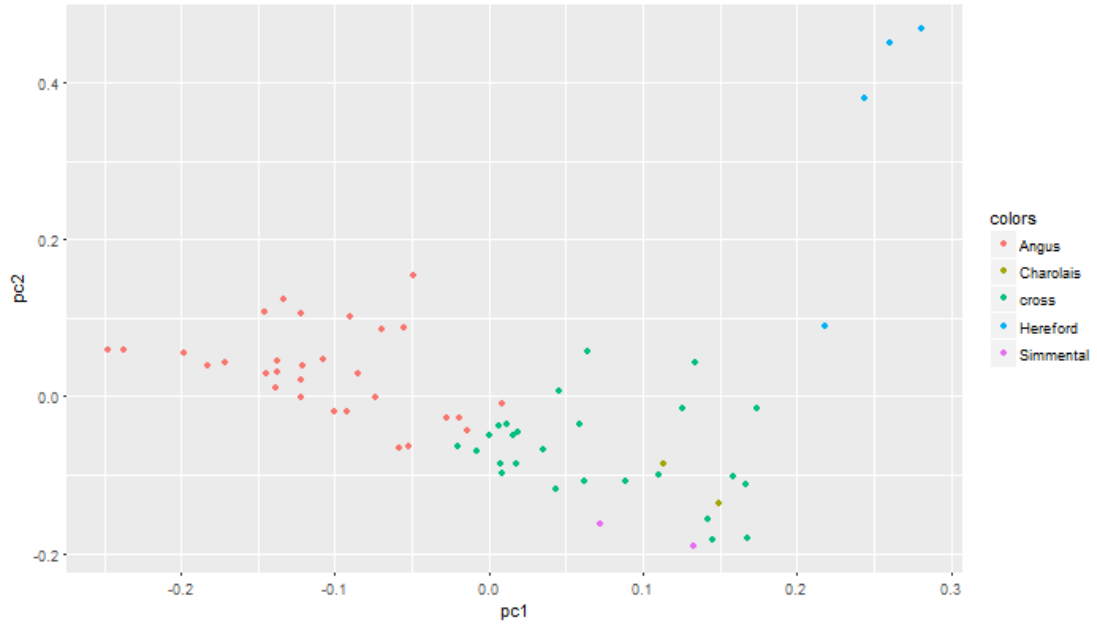
43	ARS-BFGL-BAC-11714	11	52155944	0.002427	0.930329285
44	ARS-BFGL-NGS-112489	2	72632052	0.002473	0.930329285
45	Hapmap53690-rs29026074	24	53195202	0.00248	0.930329285
46	ARS-BFGL-NGS-19663	26	43933332	0.002539	0.930329285
47	Hapmap41658-BTA-83101	2	114775268	0.002577	0.930329285
48	ARS-BFGL-NGS-28431	29	50361506	0.002578	0.930329285
49	ARS-BFGL-NGS-112197	13	80563915	0.00258	0.930329285
50	ARS-BFGL-NGS-109974	22	8207459	0.002632	0.930329285

Table 2.6. Carbohydrate metabolism related RefSeq genes (60).

Categories	Diseases or Functions Annotation	*p*-Value	Genes
Carbohydrate Metabolism, Small Molecule Biochemistry	conversion of D-glucose	0.0000635	*FABP4,INS,MAP4K4,NRIP1*
Carbohydrate Metabolism, Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry	quantity of phosphatidylethanolamine	0.000255	*ABCA4,PLA2G3,SLC27A4,UGCG,XBP1*
Carbohydrate Metabolism	quantity of carbohydrate	0.00191	*ABCA4,ALX3,CD44,CSF1,DGAT2,DGKQ,DSE,FABP4,FABP5,FMO5,FOXO1,GHR,HRH3,IDUA,IGF2,INPP5K,INS,IRS2,LPIN1,MGAT5B,MTMR3,NRIP1,P2RY2,PIP5KL1,PLA2G3,PNPLA2,PRKAB2,RIMS2,SCT,SCTR,SLC27A4,SLC5A1,STK40,TERF2IP,TGFB1,TGFBR2,UGCG,XBP1*
Carbohydrate Metabolism, Molecular Transport, Small Molecule Biochemistry	uptake of 2-deoxyglucose	0.00213	*ADRA1D,GHR,INPP5K,INS,IRS2,MYO1C,NRIP1,PRKAB2,SLC1A2,SLC27A4,WDFY2*
Carbohydrate Metabolism	synthesis of carbohydrate	0.00378	*ADRA1D,B3GNT8,CHST15,CSF1,CSGALNACT1,DGAT2,DGKQ,DPM2,DSE,ETNK2,FABP4,FABP5,FOXO1,IGF2,IL1R2,INPP5K,INS,IRS2,ITGAV,ITGB1,LRP6,NTSR2,P2RY2,P2RY6,PIGG,PIK3C2B,PISD,ST6GALNAC1,TGFB1,XBP1*
Carbohydrate Metabolism	metabolism of carbohydrate	0.00464	*ADRA1D,B3GNT8,CD44,CHST15,CSF1,CSGALNACT1,DGAT2,DGKQ,DPM2,DSE,ETNK2,FABP4,FABP5,FOXO1,FPGT,FUT9,GM2A,IDUA,IGF2,IL1R2,INPP5K,INS,IRS2,ITGAV,ITGB1,LPIN1,LRP6,NTSR2,P2RY2,P2RY6,PGM2,PIGG,PIK3C2B,PISD,PITPNA,PLA2G3,ST6GALNAC1,STK40,TALDO1,TGFB1,XBP1*

Figure 2.1 (A, B). Population structure of Group I (A) and Group II (B) beef cattle captured by top two PCs.

A



B

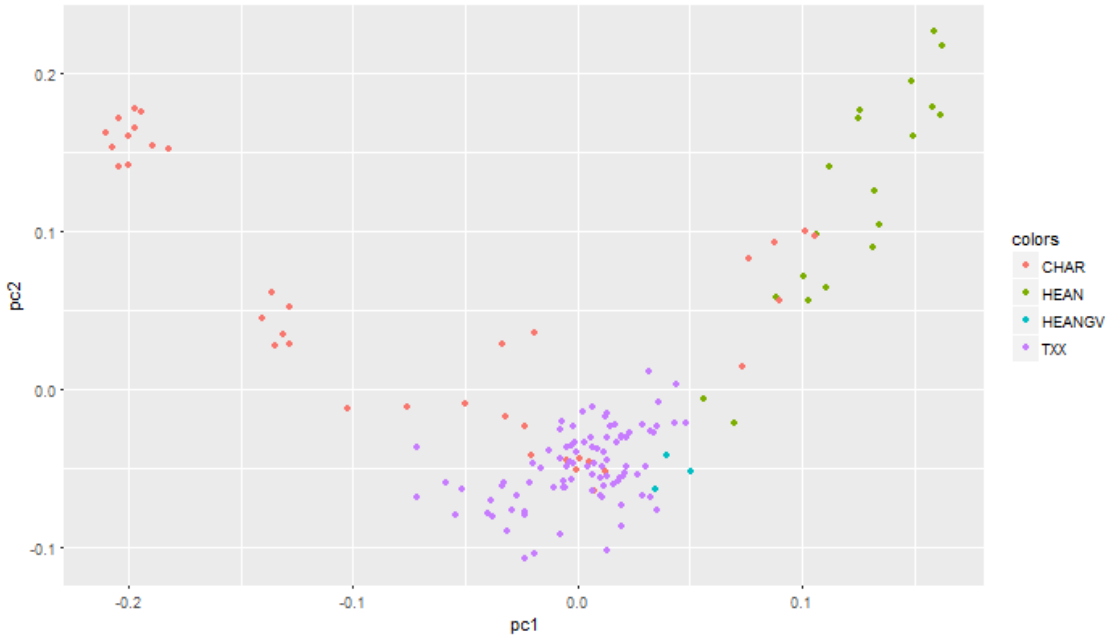


Figure 2.2 (A, B, C). Manhattan plot of all SNPs for Group I, Group II and Combined Groups. Blue line: $p = 0.01$, red line: $p = 0.001$, yellow line: $p = 0.0001$.

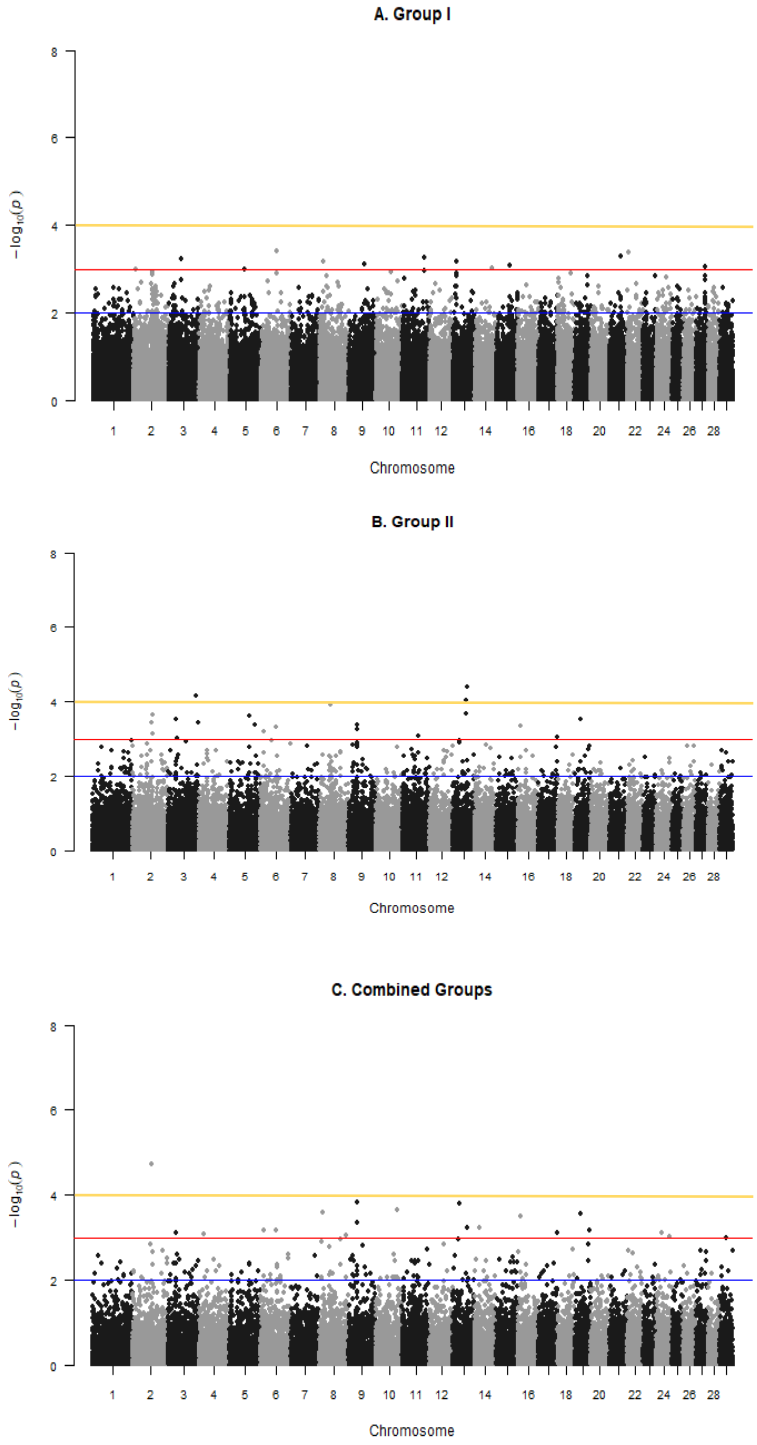


Figure 2.3. Molecular and cellular functions of the 708 RefSeq genes harboring the top 150 SNPs.

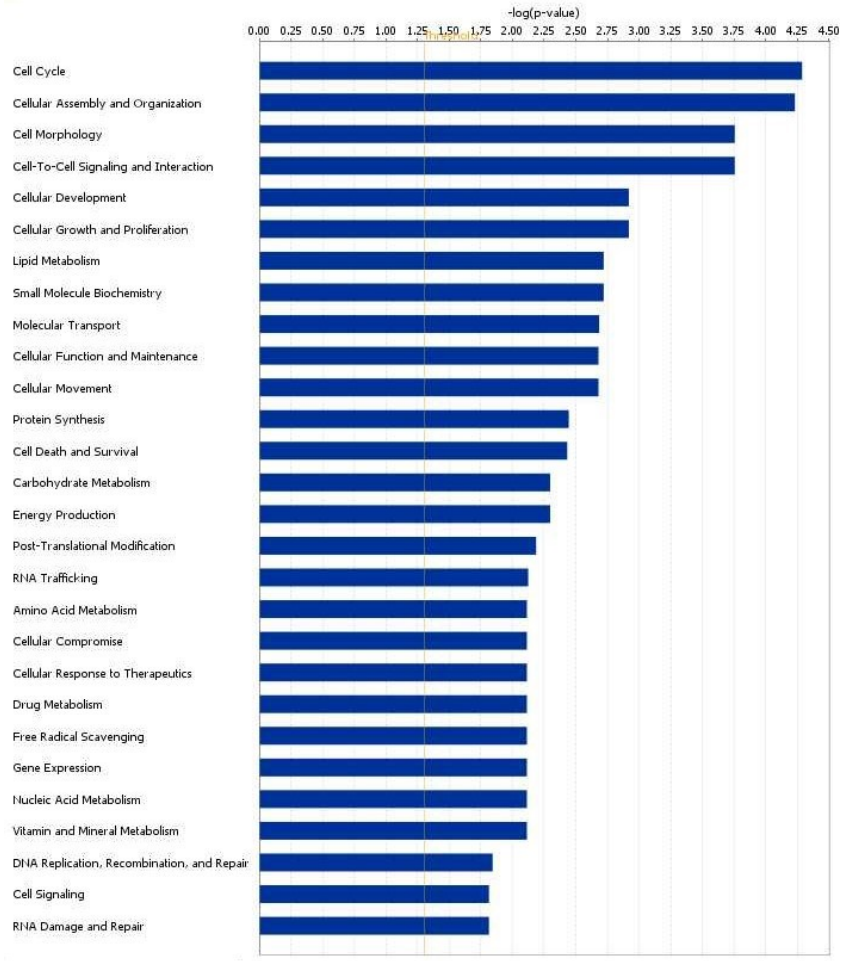


Figure 2.4. Gene network of quantity of carbohydrate, metabolism of carbohydrate and synthesis of carbohydrate (64 genes).

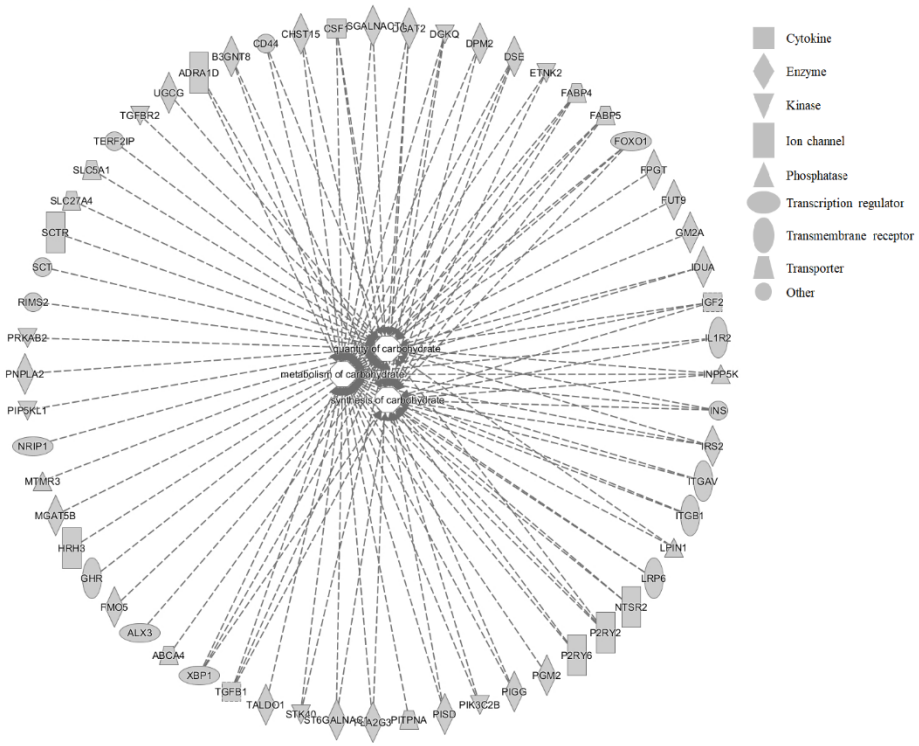
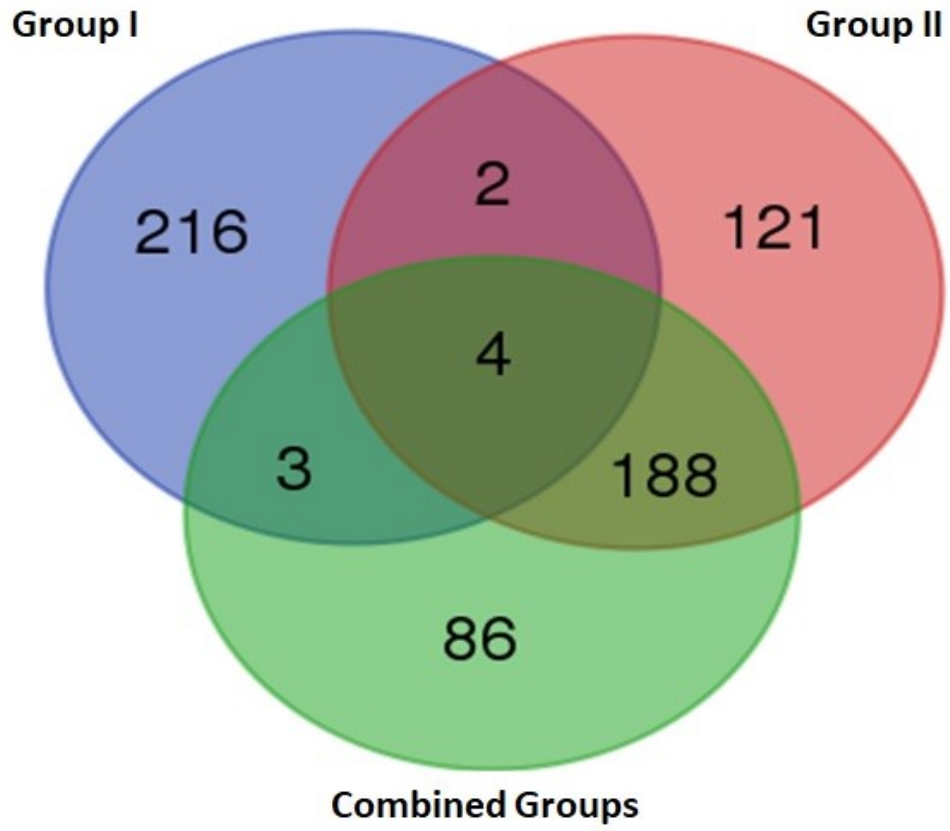


Figure 2.5. Venn Diagram of Ensembl RefSeq genes harboring the top 50 SNPs in Group I, Group II and Combined Groups.



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3. Genetic parameter estimation for sensory traits in longissimus muscle and their association with pH and intramuscular fat in pork chops

3.1. Introduction

Consumers' willingness to pay more when re-purchasing pork chops is driven by past eating satisfaction, which is determined by anticipated meat juiciness, tenderness, and flavor (Sanders et al. 2007; Mateescu et al. 2015). In beef research, according to National Consumer Retail Beef Study (Savell et al. 1987; Savell et al. 1989), meat quality attributes flavor, tenderness, and juiciness lead the consumer purchasing-decision process. In pork, these same meat quality attributes (tenderness, juiciness and flavor) are considered as important factors related to the overall sensory quality of pork chops (Goddard et al. 2012). Sensory evaluation, a scientific method accepted by many researchers, is a method using human senses, like sight, smell, touch, taste, and hearing, to evaluate products (Stone and Sidel 2004). Sensory methods have been widely used (Ophuis 1994; Keeton 1983; Brewer et al. 2001; Nam et al. 2009) to assess the quality of pork and/or pork products, contributing to the precision of subjective evaluation of meat quality characteristics and to better understanding consumer desires and meaures of satisfaction.

After slaughter, skeletal muscle glycogen is metabolized into lactic acid and hydrogen ions, with hydrogen ions being the main source of early post mortem muscle pH decrease (Tarrant 1989) and the ultimate meat pH (24 h). The meat pH is considered as a main factor influencing pork quality through the denaturation of proteins and the changes of meat color and water-holding capacity (Offer 1991; Bidner et al. 2004). According to Moeller et al. (2010), muscle ultimate pH values are associated with meat sensory traits such as tenderness and juiciness. Another major factor influencing the sensory traits of meat is the intramuscular fat content. Based on previous reports (Fortin et al. 2005; Przybylski et al. 2005), intramuscular fat,

which is the fat located between skeletal muscle fiber bundles, can significantly determine meat sensory attributes by influencing consumer's satisfaction at the point of purchase (Brewer et al. 2001; Fernandez et al. 1999; Fortin et al. 2005), as well as at the point of consumption of the meat (Brewer et al. 2001; Wood et al. 2004; Fortin et al. 2005).

There are many genetic studies related to pork meat quality (Salas and Mingala 2017; Żak and Pieszka 2009; De Vries et al. 1994; Lee et al. 2014; Liu et al. 2015), but few studies have focused on how genetics are related to meat sensory characteristics (Pommier 2004; Martel et al. 1988). Although correlations between pork sensory and pig carcass and pork quality are in the literature (Huff-Lonergan et al. 2002), there has been limited research relating both meat pH and crude fat content with eating satisfaction in addition to their genetic correlations with pork sensory attributes (Malek et al. 2001). Given the importance of improving meat quality and pork eating satisfaction, which has been addressed by the intensive selection of the swine industry (Lonergan et al. 2001), additional studies on pork sensory traits are needed to better understand their association with meat pH and intramuscular fat, and explore the possibility of genetically improving pork eating satisfaction, and hence, benefit the consumers as well as the meat industry.

This study aims to examine the influence of intramuscular pH and fat content on pork chop sensory acceptability. It also aims to estimate the heritability of pork sensory attributes as well as the phenotypic and genetic correlations of important sensory traits with pH and intramuscular fat content. The long-term objective of this study is to explore the possibility of genetically improving the eating quality of pork in the future.

3.2. Materials and methods

The animals used in this study were owned and raised by commercial pork producers. The proposed work was reviewed by the University of Alberta Animal Care and Use Committee

and considered Category A (little of no animal manipulation) and no formal ethics approval was required. No other specific permissions were required for the work as the animals were produced as part of commercial pig breeding operations and cared for according to the Canadian Quality Assurance Program, see http://www.cqa-aqc.com/resources-materials-e.php, which includes attention to animal health and well-being and is in line with the Canadian Council on Animal Care (Ofert et al. 1993) guidelines. The health of the animals was assessed daily and in the case of severe injury or when pigs failed to respond to treatment, they were humanely euthanized. Human participation in the sensory panels used in this study was also approved by a research ethics committee at the University of Alberta (PRO00029684).

3.2.1. Animals and their management

A total of 784 crossbred commercial pigs from two companies (349 from Hypor and 435 from Genesus) were used for the present study. All animals were from a typical Canadian three-way cross consisting of a Duroc sire line mated to a F1 hybrid female (Landrace × Large White). Pedigree information of all the animals was available, and there were 120 sires in total. All pigs in both populations were fed *ad libitum* and were sent to the same processing plant at a target live weight of approximately 115 kg. Details of the pigs and their production conditions were reported in a previous publication (Miar et al. 2014).

3.2.2. Meat pH and intramuscular crude fat measurement

For all the pork chop samples, fresh meat pH was measured within 24 h post-mortem. Briefly, a chop of the *longissimus dorsi* (loin) muscle at the 10th rib of the right carcass side was collected and bloomed for a minimum of 15 min at room temperature and the pH measurement was taken at two different locations on the chop using an Oakton pH/Ion 510 Bench pH/Ion/mV meter with a Kniphe electrode (double junction pH electrode) inside a Kniphe sheath with a stainless steel tip and the average from these two locations was used for final statistical analysis.

Prior to pH measurement, the electrode was calibrated with commercial pH buffers (pH 4.01 and 7.01) using a two-point calibration method.

For intramuscular crude fat content measurement, two pork loin sections were removed at carcass fabrication approximately 24 h post mortem and frozen overnight prior to shipping to the University of Alberta. One rib section (approximately 3 ribs) was thawed for 72 h at 4 °C prior to the m. *longissimus thoracis et lumborum* (LT) being cut into chops. One LT chop was used for estimation of intramuscular fat content, and was trimmed of epimysium and then cubed before being weighed, frozen at -20 °C, and then lyophilized. The cubed sample was weighed again following lyophilization for estimation of moisture loss, and ground to a powder in a stainless steel blender with 2 to 3 pellets of dry ice before being stored at -20 °C in a plastic Whirlpak™ bag until further analysis. AOAC International (2000) methods and the SoxtecTM 2050 apparatus (FOSS analytical, Soxtec) were used to measure intramuscular crude fat content (960.39) of each sample. Briefly, about 2 g of ground, freeze-dried LT muscle were weighed and placed into a cellulose extraction thimble of known weight and fat extracted using petroleum ether as the solvent. Duplicate analyses were performed on each sample and the average was used as the final value for statistical analyses.

3.2.3. Sensory evaluation

In the present study, four samples were used in each sensory evaluation session, one each representing normal pH (pH > 5.5), high fat (IMF > 2%), low pH (pH < 5.5), low fat (IMF < 2%), low pH, high fat, and normal pH and low fat product, and were evaluated by four panelists, with each panelist tasting each of the samples. The final score of each sample was the average value given by the four panelists. Samples within each treatment were assessed in order of slaughter. Two days before each sensory panel, samples were removed from storage at approximately -20 °C and thawed at 4 °C for 24 h. Before cooking, pork samples were cut into 2.54 cm thick

chops after the removal of subcutaneous fat, bone and connective tissue. Electric grills (set at 177 °C) were used to cook the samples for about 3 min each side to make sure the internal temperature of the meat reached 71 °C. Then the samples were placed into a Bain Marie at 65 °C until served to panelists. Just prior to serving to panelists, each chop was cut into 2 x 1 x 1 cm cubes, placed in a small foam cup with a lid labeled with a randomly generated three-digit identifying number, and served to panelists with unsalted crackers and room temperature water to cleanse the palate between samples.

Panelists for this study were consumers from the University of Alberta population. At each session, four panelists evaluated samples from four different animals. All the sensory evaluation sessions were performed in a sensory testing room with fluorescent lighting where panelists evaluated samples in individual booths. All the panelists evaluated pork chops for texture, juiciness and flavor using different scales. For overall texture, flavor and overall acceptability of the sample, the scale ranged from 1 = dislike extremely to 9 = like extremely; for texture, the scale ranged from 1 = much too tough to 5 = much too tender; for juiciness, the scale ranged from 1 = much too dry to 5 = much too juicy; for similar to the ideal, the scale ranged from 1 = completely different to 10 = matches to my ideal. The average score of all panelists for each animal (four panelists evaluated each sample) was used in final statistical analysis.

3.2.4. SNP Genotyping and quality control

Genomic DNA of 685 animals (subset of 784 animals) was extracted using tissue samples following the DNA extraction instructions (Thermo Fisher Scientific Ltd., Ottawa, ON, Canada) and was genotyped by Delta Genomics (Edmonton, AB, Canada) using Illumina PorcineSNP60 V2 Genotyping Beadchip which in total features 61565 Single Nucleotide

Polymorphisms (SNPs). All SNPs with a missing rate higher than 10% or a minor allele frequency (MAF) lower than 5% were removed, with 40165 SNPs passing quality control.

3.2.5. Statistical analyses

All phenotypic records outside the mean ± 3 standard deviations (SD) were considered as outliers and were treated as missing values in the analysis. After data trimming, the data followed a normal distribution tested using R/stats (R Core Team, 2013). To understand the influence of muscle pH and intramuscular fat on pork chop sensory acceptability, the samples were divided into four groups based on muscle pH value and intramuscular fat level: G1 (low pH and low fat, n = 104), G2 (low pH and high fat, n = 275), G3 (high pH and low fat, n = 137) and G4 (high pH and high fat, n = 268), with the criteria of low pH < 5.5, high pH ≥ 5.5, low fat < 2%, and high fat ≥ 2%. To test the influence of pH and intramuscular crude fat content influence on pork sensory acceptability, a 2 (high, low fat) × 2 (high, low pH) factorial was analyzed using a two-way ANOVA followed by Tukey's test for pairwise comparisons using R Statistical Package (R Core Team, 2013). Pearson correlations of pH, crude fat and sensory measurements were calculated using R/stats. Principal components of sensory measurements were generated by R/devtools, and PCA1 (first principal component) was used to represent all the six sensory measurements as an index trait.

The significances of the factors which may influence pork sensory scores, including gender, company, fat content, sensory evaluation date, animal age, days between slaughter and sensory evaluation were tested using a generalized linear model with R/stats (R Core Team 2013) and only the significant factors (gender, company and fat content) were considered in the following analysis. There were no significant interactions among the factors and hence they were not further considered in the model.

Theoretically, better estimation of genetic parameters can be obtained using high-density SNP markers compared with using the pedigree information, because high-density genotyping is being able to identify actual identical-by-state genes shared by older ancestors, whereas pedigree information is not (Forni et al. 2011), and it characterizes average genetic relationship between relatives. And in the present study, only 60k genotype information is available, so for comparison, both genetic relationships based on genotypes and pedigree were used for the estimation of variance and covariance components. Variance and covariance components of pork sensory traits were estimated using a univariate animal model in ASReml (Gilmour et al. 2015) as shown below:

$$\mathbf{Y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$$

where **Y** is the vector of phenotypes (pH, intramuscular fat and sensory measurements), **b** is the vector of fixed effects, **a** is the random additive genetic effects with [u ~ N (0, $\mathbf{A}\sigma_a^2$] (**A** is the relationship matrix that was constructed based on pedigree information. When animal genotype information is used to estimate heritability, **A** was replaced by **G** matrix generated using SNP information with the method of VanRaden 2008), σ_a^2 is the polygenetic additive variance, **e** is the vector of residual errors with a distribution of [e ~ N (0, $\mathbf{I}\sigma_e^2$)], where **I** is the identity matrix and σ_e^2 is the residual variance. **X** and **Z** are the incidence matrices for **b** and **a**, respectively. The factors in the model included company (Hypor and Genesus) and gender (male and female) as fixed effects, and and crude fat content as a covariable. Phenotypic variance was calculated as $\sigma_p^2 = \sigma_a^2 + \sigma_e^2$, and the heritability was calculated as $h^2 = \sigma_a^2/\sigma_p^2$.

Phenotypic, genetic and environmental variances and co-variances were estimated using a bivariate animal model in ASReml (Gilmour et al. 2015). The bivariate analysis was performed between pH and crude fat with all pork sensory measurements using the following model:

$$\begin{bmatrix} \boldsymbol{y_1} \\ \boldsymbol{y_2} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{X_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{b_1} \\ \boldsymbol{b_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{Z_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{a_1} \\ \boldsymbol{a_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{e_1} \\ \boldsymbol{e_2} \end{bmatrix}$$

Where $\boldsymbol{y_1}$ and $\boldsymbol{y_2}$ are vectors of phenotypic values for any two paired traits considered in the model; $\boldsymbol{b_1}$ and $\boldsymbol{b_2}$ are vectors of fixed effects for trait 1 and trait 2, respectively; $\boldsymbol{a_1}$ and $\boldsymbol{a_2}$ are vectors of random additive genetic effects; $\boldsymbol{e_1}$and $\boldsymbol{e_2}$ are vectors of random residual effects; and **X** and **Z** are known design matrices for fixed effects and random additive genetic effects, respectively. Multivariate normal distributions were assumed for the random vector **a**, with means equal to **0**, which leads to E(y) = **Xb**. The variance - covariance matrix for the random effects is described as below:

$$\text{var}\begin{bmatrix} a_1 \\ a_2 \\ e_1 \\ e_2 \end{bmatrix} = \begin{bmatrix} \boldsymbol{A}\sigma_{a_1}^2 & \boldsymbol{A}\sigma_{a_1a_2} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{A}\sigma_{a_1a_2} & \boldsymbol{A}\sigma_{a_2}^2 & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{I_{n_e}}\sigma_{e_1}^2 & \boldsymbol{I_{n_e}}\sigma_{e_1e_2} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{I_{n_e}}\sigma_{e_1e_2} & \boldsymbol{I_{n_e}}\sigma_{e_2}^2 \end{bmatrix},$$

where $\sigma_{a_1}^2$ and $\sigma_{a_2}^2$ are the additive genetic variance for trait 1 and trait 2, respectively, and $\sigma_{a_1a_2}$ is the genetic covariance between the two traits; **A** is the additive genetic relationship matrix constructed from the pedigree, which consisted of 8,372 animals in total in the pedigree file; $\sigma_{e_1}^2$ and $\sigma_{e_2}^2$ are the residual variance for trait 1 and trait 2, respectively, and $\sigma_{e_1e_2}$ is the residual covariance between the two traits; and $\boldsymbol{I_{n_e}}$ is the identity matrix with dimension $n_e \times n_e$, in which n_e is the number of animals with records. Variance and covariance components were estimated by restricted maximum likelihood as implemented in ASReml (Gilmour et al. 2015). Phenotypic variance and covariance were calculated as $\sigma_p^2 = \sigma_a^2 + \sigma_e^2$ and $\sigma_{p_1p_2} = \sigma_{a_1a_2} + \sigma_{e_1e_2}$,

respectively. The phenotypic and genetic correlations were then estimated as $r_p = \sigma_{p_1 p_2} / \left(\sigma_{p_1}^2 \sigma_{p_2}^2\right)^{1/2}$ and $r_a = \sigma_{a_1 a_2} / \left(\sigma_{a_1}^2 \sigma_{a_2}^2\right)^{1/2}$, respectively.

3.3. Results

3.3.1. Influence of muscle pH and intramuscular fat on pork chop sensory acceptability

Two-way ANOVA showed that for all sensory scores of pork chops there was no effect of intramuscular fat or pH ($p > 0.05$) on all the sensory measurements, with the exception of pH on texture (Table 3.1) (Supplementary Table 3.1). Meat pH was lowly correlated with all the sensory measurements (r = 0.05 to 0.12), and fat was also lowly correlated with all sensory measurements (r = 0.16 to 0.22). Correlations between pH and sensory measurements were lower than those between fat and sensory measurements (Table 3.3).

3.3.2. Heritability

The variance components and heritability estimates of all sensory traits are shown in Table 3.2. Low heritability was estimated for all the sensory measurements using either pedigree or genotype information. For all the sensory measurements, the estimated heritability using **A** matrix were larger than using **G** matrix.

In order to check the possible influence of collinearity of the sensory measurements, and the association of sensory traits with pH and intramuscular fat, Pearson correlations of all the measurements were calculated. The six sensory traits were highly correlated with each other (Table 3.3). The principal components shown in Figure 3.1 confirmed that these sensory measurements were tightly related. An index named PCA1 (first principal component) was generated to represent all the six sensory measurements as one trait, and the correlation between pH and PCA1 was 0.09 ($p = 0.02$), and between intramuscular fat and PCA1 was 0.21 ($p < 0.0001$) (not included in Table 3.2). The heritability of PCA1 was 0.10 ± 0.06 and 0.05 ± 0.04 according to **A** and **G** matrix, respectively.

3.3.3. Genetic correlations

Genetic and phenotypic correlations of sensory measurements with pH and intramuscular fat using pedigree information were presented in Table 3.4. Meat pH had low to moderate genetic correlation with sensory measurements ranged from 0.16 to 0.39. Intramuscular fat had moderate to high genetic correlation with sensory measurements (0.37 - 0.54). The genetic correlation of PCA1 with pH and intramuscular fat was about 0.31 ± 0.19 and 0.51 ± 0.20, respectively. Meat pH had very low phenotypic correlations with all the sensory measurements. Intramuscular fat had low to moderate phenotypic correlations with all the sensory measurements.

3.4. Discussion

3.4.1 Influence of ultimate pH and intramuscular fat on meat sensory acceptability

Meat ultimate pH value has been used as a primary postmortem indicator of pork quality (Offer 1991) since its first introduction in pork research to diagnose PSE carcasses in 1959 (Wismer-Pedersen 1959). After that, a substantial volume of research has been performed to elucidate the influence of ultimate pH on meat quality and meat tenderness specifically (Bouton et al. 1973; Jeremiah et al. 1991; Purchas et al. 1993; Guignot et al. 1994), with regard to the linearity or curvilinearity of the relationship between meat pH and tenderness. According to Huff-Lonergan et al. (2002), meat pH was significantly ($r = 0.27$, $p = 0.0001$) correlated with tenderness score, a low correlation that roughly agrees with the result of the present study where a low correlation between meat pH and sensory texture was obtained ($r = 0.12$, $p < 0.05$) (Table 3.3).

For other sensory measurements, the correlations were very low (Table 3.3), and the sensory panel was not able to differentiate pork of the different pH groups in either low or high fat groups in terms of its eating satisfaction (Table 3.1), suggesting that meat pH has no effect on pork chop sensory characteristics. Lonergan et al. (2007) found that increased intramuscular fat

tended to increase sensory perception of tenderness in pork with an intramuscular pH of 5.5 to 5.65, contrasting with the present study. Lonergan et al. (2007) did not, however, freeze their pork prior to sensory analysis, while that of the present study was frozen prior to analysis and pH analysis. This disparity suggests that freezing pork reduces or eliminates the influence of pH on the integrity of myofibrillar proteins, removing the effect of intramuscular fat on the perception of tenderness.

For the influence of intramuscular fat on eating satisfaction of pork chops, different conclusions have been presented by previous studies. Fortin et al. (2004) reported that intramuscular fat has significant influence on softness, initial tenderness, chewiness, and flavor intensity of pork. According to Jeremiah et al. (2003), intramuscular fat has direct influence on meat juiciness and flavor, and indirect influence on meat tenderness. In heavily marbled muscles, intramuscular fat, located between muscle fiber fascicules, can disrupt the structure of endomysium, separate and dilute perimysial collagen fibres, thus contributing to meat tenderness (Nishimura et al. 1999; Jeremiah et al. 2003; Hocquette et al. 2010). On the other hand, Rincker et al. (2007) reported that there was no strong correlation between intramuscular fat and meat tenderness, juiciness or flavor, and according to Van Laack et al. (2001) and Channon et al. (2004), there were no or very small associations between intramuscular fat content and meat eating quality. According to Lonergan et al. (2007), lipid content had a small positive effect on pork texture and tenderness with pH between 5.50 and 5.80, but not at a lesser or higher pH, suggesting the importance of defining meat pH range when evaluating the influence of intramuscular fat on pork sensory traits. The possible reasons for these inconsistent results about the influence of meat pH and intramuscular fat on pork eating satisfaction may be due to the difference of genetic background, chop sample positions on the loin, sample sizes, meat pH,

and/or freezing. The present study used 784 typical Canadian three-way crossbred commercial pigs and pork chops from the same position on the loin from each animal for the sensory evaluation session. Our results showed that intramuscular fat content had no significant influence on all the sensory measurements and the sensory panel was not able to differentiate pork of the different fat groups in either low or high pH groups in terms of its eating satisfaction (Table 3.1). However, different conclusion may be made if different grouping strategy using intramuscular fat content level is used to group the samples, as well as the classification of meat pH values like the one reported by Lonergan et al. (2007), which may be worthy of doing in the future.

3.4.2. Heritability of sensory measurements

Until now, most of the reported heritability estimates were associated with objective measurements of meat quality traits, such as meat pH, shear force, colour and drip loss (De Vries et al. 1994; Suzuki et al. 2005; Gilbert et al. 2007). There is no scientific report related to the genetic parameter estimates forpork sensory attributes and only a few reported for beef sensory acceptability (Riley et al. 2003; Mateescu et al. 2015). Based on these results, we may consider beef and/or pork sensory attributes as low heritable traits with the heritability ranging from 0.02 to 0.21. Our present study further confirmed that pork sensory attributes are lowly heritable (≦ 0.13, Table 3.2). In the present study, the heritability estimates of sensory traits are very low and similar to each other, indicating the possible existence of collinearity among them. The description of these sensory measurements as shown in Materials and Methods also reflected the similarity of the traits. Hence, we basically estimated the same parameter if we treated the measurements separately as different traits. To avoid the influence of collinearity, PCA1 was generated by using the values of the six sensory measurements to represent all the sensory attributes in one trait. The low heritability of PCA1 (0.10 from **A** and 0.05 from **G**) further indicated that pork sensory was low heritable.

Larger additive genetic variances were detected using pedigree (**A** matrix) than when using genotype (**G** matrix) information. When using the **A** matrix, average additive genetic relationship between animals is estimated based on the pedigree information, and deeper, more accurate pedigree information can generate better (more precise) results. In this study, a deep pedigree which traced back about 9 ancestral generations comprising 8,373 individuals was used, which was able to capture the precise genetic relationships among the animals considering many generations. For the **G** matrix, DNA makers (SNPs) were used to calculate the average genetic distance between the animals. Usually, high density SNPs spanning the whole genome are expected to construct a more accurate relationships among animals than using pedigree (Gilmour, 2015; Zhang et al. 2017). However, for complex quantitative traits especially the low heritable ones (such as the sensory traits in this study), they are genetically influenced by a large number of genes/markers with very small effect contributed by individual marker, plus they are easily influenced by environment. Therefore, more trait associated markers/SNPs are available in the panel, more accurate genetic variance can be captured by using this SNP panel. In the present study, larger genetic variance was estimated using pedigree than using genotypes which is most likely due to that the SNPs we used were not enough to capture genetic variance of sensory traits, compared using the deep pedigree. For this particular reason, the genetic and phenotypic correlations of sensory traits with pH and intramuscular fat were estimated only using **A** matrix.

3.4.3 Genetic and phenotypic correlations of meat sensory attributes with pH and intramuscular fat

Because of the relatively small sample size and low heritability of panel sensory attributes, the estimated correlations of sensory traits with pH and intramuscular fat in this study should be interpreted with caution. Moderate to low genetic correlations were detected between pH and sensory traits, ranging from 0.16 to 0.39 (Table 3.4). However, no research was found

that related pork intramuscular pH and sensory attributes genetically. The most related research reported genetic correlations of ultimate pH and meat quality traits like meat colour and drip loss, and these traits were moderately to highly genetically correlated (Hovenier et al. 1992; Knapp et al. 1997; Hermesch et al. 2000). For intramuscular fat, moderate to high genetic correlations with sensory measurements were estimated, ranging from 0.37 to 0.54. Most previous scientific studies corroborate our results, reporting that intramuscular fat content has a positive correlation with meat eating satisfaction (Fernandez et al. 1999a; Fernandez et al. 1999b; Fortin et al. 2004; Daszkiewicz et al. 2005), while Lonergan et al. (2007) reported that intramuscular lipid content was not associated with pork sensory texture, tenderness, or chewiness when meat pH was lower than 5.50 or higher than 5.80, suggesting that intramuscular fat can influence pork sensory characteristics only at intermediate meat pH. As the heritability for these sensory traits was low, it might be very difficult to improve these traits by direct selection, and selection for these traits is also complicated by the difficulty associated with collecting these phenotypes for a large number of animals. Meat pH and intramuscular fat are usually two common breeding goal traits to improve meat quality. Favorable genetic correlations of sensory measurements with pH and intramuscular fat were found in this study, which indicates improving pH and intramuscular fat can help to improve sensory traits.

3.5. Conclusions

In the present study, all pork sensory measurements had relatively low heritability, suggesting that direct selection on these traits might be ineficienct. Positive genetic correlations between sensory measurements and pH and intramuscular fat were found, indicating that selection for intermediate pH and high intramuscular fat can help to increase sensory scores. However, as the genetic correlations were moderate to low, increase in pork sensory scores through selection for loin pH and intramuscular fat content would be slow.

Table 3.1. Least squares means and standard errors of pork sensory attributes.

Measurements/Groups	**G1**(Low pH < 5.5, low fat < 2%, n=104)	**G2**(low pH < 5.5, high fat > 2%, n=275)	**G3**(High pH ≥ 5.5, low fat < 2%, n=137)	**G4**(High pH ≥ 5.5, high fat > 2%, n=268)
Overall Texture	5.64 ± 0.10	5.72 ± 0.06	5.59 ± 0.09	5.68 ± 0.06
Texture	2.44 ± 0.05	2.47 ± 0.03	2.35 ± 0.04	2.41 ± 0.03
Juiciness	2.44 ± 0.04	2.43 ± 0.03	2.42 ± 0.04	2.45 ± 0.03
Flavour	5.71 ± 0.09	5.87 ± 0.05	5.78 ± 0.07	5.86 ± 0.05
Overall Opinion	5.52 ± 0.10	5.68 ± 0.06	5.53 ± 0.08	5.65 ± 0.06
Similar to Ideal	6.23 ± 0.11	6.40 ± 0.07	6.33 ± 0.10	6.41 ± 0.07

Note: Different superscripts in each row denote significant difference ($p < 0.05$) from Tukey's test.

Table 3.2. Variance components and heritability estimates (±SE) of sensory measurements using either pedigree (**A** matrix) or genotype (**G** matrix) information.

Measurements	A matrix			G matrix		
	Heritability	σ_a^2±SE	σ_e^2±SE	Heritability	σ_a^2±SE	σ_e^2±SE
Overall Texture	0.13±0.06	0.14±0.07	0.93±0.08	0.09±0.05	0.09±0.04	0.93±0.09
Texture	0.07±0.06	0.14±0.01	0.19±0.01	0.06±0.04	0.01±0.01	0.19±0.02
Juiciness	0.08±0.05	0.01±0.01	0.16±0.01	0.02±0.02	0.00±0.00	0.17±0.01
Flavor	0.02±0.05	0.02±0.04	0.72±0.05	0.00±0.02	0.00±0.01	0.75±0.05
Overall Opinion	0.09±0.06	0.08±0.05	0.83±0.06	0.05±0.04	0.04±0.03	0.85±0.07
Similar to Ideal	0.12±0.06	0.15±0.08	1.07±0.09	0.06±0.04	0.07±0.04	1.08±0.09
PCA1	0.10±0.06	0.38±0.23	3.51±0.27	0.05±0.04	0.20±0.14	3.60±0.30

Table 3.3. Pearson correlations between pH, intramuscular fat and pork sensory measurements.

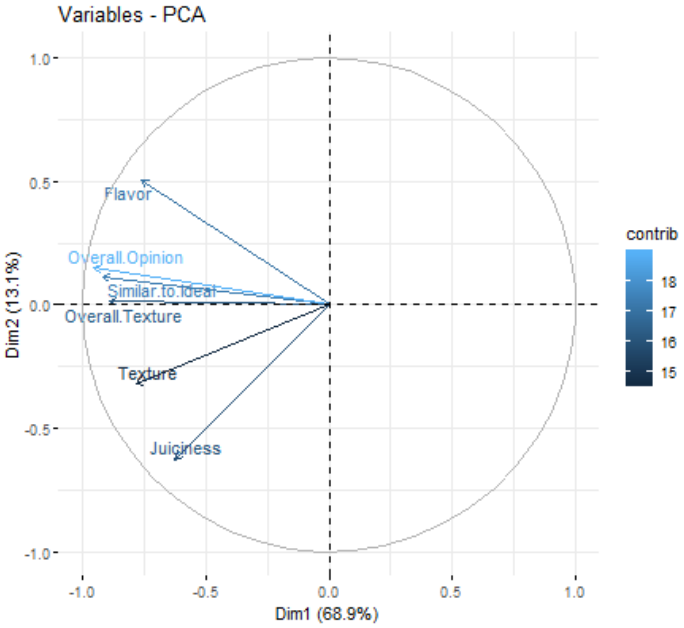
	pH	Intramus-cular Fat	Overall Texture	Texture	Juiciness	Flavor	Overall Opinion	Similar to Ideal
pH	1	0.05	0.05	0.12**	0.10**	0.06**	0.08*	0.08*
Intramuscular Fat		1	0.16***	0.16***	0.18***	0.16***	0.22***	0.20***
Overall Texture			1	0.72***	0.45***	0.59***	0.84***	0.77***
Texture				1	0.49***	0.39***	0.66***	0.64***
Juiciness					1	0.31***	0.52***	0.51***
Flavor						1	0.77***	0.71***
Overall Opinion							1	0.88***
Similar to Ideal								1

Note: Significance levels were set as: ***$p < 0.001$, **$0.001 \leqq p < 0.01$, *$0.01 \leqq p < 0.05$.

Table 3.4. Genetic and phenotypic correlations (±SE) of sensory measurements with pH and intramuscular fat using pedigree information.

Measurements	Genetic Correlation		Phenotypic Correlation	
	pH	Intramuscular Fat	pH	Intramuscular Fat
Overall Texture	0.16±0.16	0.37±0.19	0.03±0.04	0.16±0.04
Texture	0.34±0.25	0.54±0.23	0.10±0.03	0.17±0.04
Juiciness	0.30±0.21	0.51±0.22	0.08±0.04	0.18±0.04
Flavor	0.39±0.37	0.48±0.54	0.03±0.03	0.16±0.04
Overall Opinion	0.34±0.21	0.47±0.22	0.04±0.04	0.23±0.04
Similar to Ideal	0.21±0.17	0.49±0.18	0.06±0.04	0.21±0.04
PCA1	0.31±0.19	0.51±0.20	0.06±0.04	0.22±0.04

Figure 3.1. Principal component analysis of pork sensory measurements.



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4. Efficacy of genetic parameter estimation of pork loin quality of crossbred commercial pigs using technological quality measurements of frozen and unfrozen product

4.1. Introduction

Meat is a major source of highly biologically available proteins and micronutrients that are necessary for physical health (William 2007). The global meat export industry has been estimated to be worth more than US$ 13 billion (Leygonie et al. 2012a) and freezing meat plays an essential role in prolonging meat shelf-life and ensuring meat safety during global transport (William 2007). Consumers commonly freeze meat after purchasing it to manage domestic meat supply and meat is also frozen by researchers to manage sample inventory and analysis volumes, despite well-known freeze-thaw-induced changes to meat quality (Leygonie et al. 2012a). Meat quality characteristics that may change as a result of freezing and thawing include meat pH, color, drip loss, Warner-Bratzler shear force and sensory properties (Deatherage and Hamm 1960; Ngapo et al. 1999; Lagerstedt et al. 2008; Vieira et al. 2009; Leygonie et al. 2012b). The effects of freezing on meat quality appear to arise from the formation of ice crystals on muscle cell and organelle membranes during freezing and the inability of water to be re-bounded by the myofilaments during thawing (Leygonie et al. 2012b). When meat is subjected to freezing and then thawing, there can be a reduction in Z-disk density and a loss of myofibril integrity (Yu et al. 2010).

The extent of changes to meat quality, especially to WHC, that occur with freezing and thawing are very much dependent on the rates of freezing and thawing. According to Yu et al. (2010), a rapid freezing rate and a slow thawing rate will have minimal effects on thaw loss, cook loss, and protein solubility, as ice crystals formed during freezing are small and water uptake during thawing is possible (Yu et al. 2010). The thermal conductivity of fat and water also are different so changes in the rate of freezing and thawing would be expected between

water-rich lean muscle and muscle with increased contents of intramuscular fat (Kumcuoglu et al. 2010).

With many years of selection for production efficiency, the quality of meat has been compromised as indicated by reduced post-mortem pH, increased Warner-Bratzler shear values, and increased moisture and protein loss (Lonergan et al. 2001). To address this, there is increased attention to meat quality improvement (Morgan et al. 1994) and substantial research related to the estimation of pork quality genetic parameters has been conducted (Cameron 1990; Lo et al. 1992; De Vries et al. 1994; Knapp et al. 1997; Sonesson et al. 1998; Suzuki et al. 2005; Gjerlaug-Enger et al. 2010; Miar et al. 2014a). However, none of these studies considered if genetic parameters estimated for meat quality traits were conducted on fresh or previously frozen and subsequently thawed meat. Considering the substantial changes possible in meat quality after freezing and thawing, we hypothesized that genetic parameters estimated from meat quality data obtained from fresh meat will be different to those estimated from data on frozen-thawed meat. The objectives of the present study were to estimate: (1) heritability of important meat quality traits measured using fresh and frozen-thawed pork; (2) phenotypic, genetic and environmental correlations of meat quality measurements of fresh and frozen-thawed pork; and (3) the effect of crude fat content on meat quality measurements in fresh and frozen-thawed pork from carcasses of commercial crossbred pigs.

4.2. Materials and methods

The animals used in this study were cared for according to the Canadian Council on Animal Care (Olfert et al. 1993) guidelines.

4.2.1. Animals and management

A total of 2027 crossbred commercial pigs from two companies (1076 samples from Hypor and 951 samples from Genesus) were obtained for this study. All animals were from a

typical Canadian three-way cross consisting of a Duroc sire line mated to a F1 hybrid female (Landrace × Large White). Pedigrees on all animals were available, and there were 120 sires. Pigs in both populations were fed ad libitum. All animals were sent to the same processing plant at a target live weight of approximately 115 kg. Details of the pigs and their production conditions were reported in a previous publication (Miar et al. 2014a).

4.2.2. Meat quality traits measurement

Carcass and fresh meat quality measurements were performed within 24 h post-mortem. For fresh meat quality measurements, a chop of the longissimus dorsi loin muscle at the 10th rib of the right carcass was collected and bloomed for a minimum of 15 min at room temperature before pH and Minolta color measurements. Fresh meat pH measurements were taken at two locations on the chop using an Oakton pH/Ion 510 Bench pH/Ion/mV meter with a Kniphe electrode (double junction pH electrode) inside a Kniphe sheath with a stainless steel tip and their average was used for the final statistical analysis. Prior to pH measurement, the electrode was calibrated with commercial pH buffers (pH 4.01 and 7.01) using a two point calibration method. Fresh pork color was described by the color system values specified by the Commission Internationale de L'eclairage (CIE) where L* is lightness, a* is redness and b* is yellowness, and values were measured using the C illuminant at four locations on each chop with a Minolta CX-10 colorimeter (Konica Minolta Sensing Inc., Japan) and the average was used for the final statistical analysis. Fresh meat drip loss was evaluated using a second loin chop that had been trimmed of fat and bone. The trimmed chop was weighed and then placed on a stainless steel grid above a tray and spaced so that none of the samples touched another. Chops were stored at 2 °C for 48 h on the stainless steel grid and tray and then re-weighed (Barton-Gade et al. 1994). The drip loss was expressed as the percentage of weight loss of the initial weight.

A portion of the remaining pork loins (4th -7th ribs) was harvested from the right side of each pig carcass, packaged under vacuum, and frozen (-20 ºC) within 24 h of exsanguination. Packaged loins were maintained frozen until prepared for frozen-thawed meat quality measurement. For the frozen-thawed meat quality measurements, the frozen pork loins were thawed for 61 h at 4 ºC. Meat pH was then measured using an Accumet AP71 Portable Waterproof pH Meter (Fisher Scientific Company, Toronto, Ontario) equipped with a glass electrode calibrated at room temperature using standards of pH 4.01 and 7.01. The pH probe was used to measure pH at three different locations within 2.5 cm of the posterior of the longissimus dorsi section, and the mean of the three pH values was used as the final value for statistical analysis. Pork color was again described by the CIE color system but with a Minolta CR-400 Chroma Meter (Konica Minolta Sensing Inc., Japan) using the D65 illuminant. A chop about 2.5 cm thick was cut from the posterior of the 4-7th rib pork loin section and bloomed for 1 h at 4 ºC prior to color measurement. Color measurements were then taken from three different locations on the surface of the bloomed muscle, and the average value for each color value was used for the final statistical analysis. For frozen-thawed pork drip loss measurement, the plastic-bag method (Honikel 1998) was used. Briefly, a 100 ± 10 g pork chop was cut transversely with the direction of muscle fibre, trimmed of epimysium, fat and bone, weighed and suspended by a metal hook in a closed plastic bag for 24 h. The bag was inflated sufficiently to prevent contact with the meat. Again, drip loss was expressed as the percentage of weight loss of the initial sample.

SoxtecTM 2050 apparatus (FOSS analytical, Soxtec) was used to measure marbling as intramuscular crude fat (960.39) of a chop from the frozen pork loin section by following AOAC International (2000) methods. Briefly, about 2 g of ground, freeze-dried longissimus dorsi

muscle were weighed and placed into a cellulose extraction thimble of known weight and fat extracted using petroleum ether as solvent. Duplicate analyses were performed on each loin and the average was used as the final value for statistical analyses.

4.2.3. Statistical analyses

Meat quality measurements from fresh pork were first calculated and presented by Miar et al. (2014b) and a subset of the data were used in this study. All phenotypic records outside the mean ± 4 standard deviations (SD) were considered as outliers (30 in total) and deleted from the data set. After data trimming, the data followed a normal distribution tested through the univariate procedure in SAS 9.3 (SAS Institute Inc.). The difference (Δ) in average color and pH between fresh and frozen-thawed samples was tested by paired t-test (dependent t-test) using SAS 9.3 (SAS Institute Inc.) with a significance level of $p < 0.0001$.

Phenotypic, genetic and environmental variances and co-variances were estimated using a bivariate animal model in ASReml (Gilmour et al. 2015). The analysis was performed for all pairwise meat quality traits between fresh and frozen-thawed pork regardless of different methods being used for drip loss because we treated them as independent traits. The analysis was performed within the fresh samples and frozen-thawed samples as well. The bivariate animal model can be described as:

$$\begin{bmatrix} \boldsymbol{y_1} \\ \boldsymbol{y_2} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X_1} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{X_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{b_1} \\ \boldsymbol{b_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z_1} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{Z_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{a_1} \\ \boldsymbol{a_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{W_1} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{W_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{c_1} \\ \boldsymbol{c_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{e_1} \\ \boldsymbol{e_2} \end{bmatrix}$$

Where $\boldsymbol{y_1}$ and $\boldsymbol{y_2}$ are vectors of phenotypic values for any two paired traits considered in the model; $\boldsymbol{b_1}$ and $\boldsymbol{b_2}$ are vectors of fixed effects for trait 1 and trait 2, respectively; $\boldsymbol{a_1}$ and $\boldsymbol{a_2}$ are vectors of random additive genetic effects; $\boldsymbol{c_1}$ and $\boldsymbol{c_2}$ are vectors of random contemporary group effects; $\boldsymbol{e_1}$and $\boldsymbol{e_2}$ are vectors of random residual effects; and **X**, **Z**, and **W** are known design matrices for fixed effects, random additive genetic effects, and random contemporary group

effects, respectively. The fixed effects included company (Hypor and Genesus) and sex (male and female), and crude fat content was fitted as a linear covariate. The random contemporary group is a combination of slaughter batch, year and growing groups during the test (~ 70 – 115 kg) with 57 levels for Genesus and 44 for Hypor. Multivariate normal distributions were assumed for the random vectors **a**, **c**, with means equal to **0**, which leads to E(y) = **Xb**. The variance-covariance matrix for the random effects is described as below:

$$\text{var}\begin{bmatrix} a_1 \\ a_2 \\ c_1 \\ c_2 \\ e_1 \\ e_2 \end{bmatrix} = \begin{bmatrix} \boldsymbol{A}\sigma_{a_1}^2 & \boldsymbol{A}\sigma_{a_1a_2} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \boldsymbol{A}\sigma_{a_1a_2} & \boldsymbol{A}\sigma_{a_2}^2 & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \boldsymbol{I}_{\boldsymbol{n_c}}\sigma_{c_1}^2 & \boldsymbol{I}_{\boldsymbol{n_c}}\sigma_{c_1c_2} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \boldsymbol{I}_{\boldsymbol{n_c}}\sigma_{c_1c_2} & \boldsymbol{I}_{\boldsymbol{n_c}}\sigma_{c_2}^2 & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \boldsymbol{I}_{\boldsymbol{n_e}}\sigma_{e_1}^2 & \boldsymbol{I}_{\boldsymbol{n_e}}\sigma_{e_1e_2} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \boldsymbol{I}_{\boldsymbol{n_e}}\sigma_{e_1e_2} & \boldsymbol{I}_{\boldsymbol{n_e}}\sigma_{e_2}^2 \end{bmatrix},$$

in which $\sigma_{a_1}^2$ and $\sigma_{a_2}^2$ are the additive genetic variance for trait 1 and trait 2, respectively, and $\sigma_{a_1a_2}$ is the genetic covariance between the two traits; **A** is the additive genetic relationship matrix constructed from the pedigree, which consisted of 8,372 animals in total in the pedigree file; $\sigma_{c_1}^2$ and $\sigma_{c_2}^2$ are the variance of contemporary group effects for trait 1 and trait 2, respectively, and $\sigma_{c_1c_2}$ is the covariance between the two traits due to the same contemporary groups; covariance between different contemporary group effects were considered 0; $\boldsymbol{I}_{\boldsymbol{n_c}}$is the identity matrix with dimension $n_c \times n_c$, in which n_c is the number of random contemporary groups; $\sigma_{e_1}^2$ and $\sigma_{e_2}^2$ are the residual variance for trait 1 and trait 2, respectively, and $\sigma_{e_1e_2}$ is the residual covariance between the two traits; and $\boldsymbol{I}_{\boldsymbol{n_e}}$ is the identity matrix with dimension $n_e \times n_e$, in which n_e is the number of animals with records. Variance and covariance components were estimated by restricted maximum likelihood. Phenotypic variance and covariance were calculated as $\sigma_p^2 = \sigma_a^2 + \sigma_c^2 + \sigma_e^2$ and $\sigma_{p_1p_2} = \sigma_{a_1a_2} + \sigma_{c_1c_2} + \sigma_{e_1e_2}$, respectively. The phenotypic and genetic

correlations were then estimated as $r_p = \sigma_{p_1 p_2} / \left(\sigma_{p_1}^2 \sigma_{p_2}^2\right)^{1/2}$ and $r_a = \sigma_{a_1 a_2} / \left(\sigma_{a_1}^2 \sigma_{a_2}^2\right)^{1/2}$, respectively. The heritability was defined as $h^2 = \sigma_a^2 / \sigma_p^2$. Heritability estimates were reported as the averages of all bivariate analyses of the trait.

4.3. Results

4.3.1. Changes in meat quality from fresh to frozen-thawed

Paired t-tests (Table 4.1) showed that the changes in measurement values from fresh to frozen-thawed samples were significant ($p < 0.0001$) for meat color (L*, a* and b*) and pH. Freezing and thawing pork appeared to darken it (decreased L* and b*, increased a*) when the mean of previously frozen chops was compared with that of the fresh samples (Table 4.1).

4.3.2. Crude fat content effect on meat quality changes

Figure 4.1 (A) and (C) showed that, with the increase of crude fat content, the changes in L* and b* with freezing and thawing both increased and then decreased. In Figure 4.1 (A), as crude fat content increased up to 4% intramuscular fat, there was an associated increased difference in mean L* value between fresh and frozen-thawed pork.

4.3.3. Heritability

The heritability estimates of meat quality measurements from fresh and frozen-thawed pork are shown in Table 4.2 and Table 4.3, and those from the fresh pork were first calculated and presented by Miar et al. (2014b). For meat color, L* and a* measured on fresh and frozen-thawed pork had low to moderate heritability. The heritability estimates newly calculated in this study for the five measurements on fresh meat were in agreement with those calculated in our previous study (Miar et al. 2014b) and other reports (Van Wijk et al. 2005; Gjerlaug-Enger et al. 2010), except a slight difference for b* (0.10) and pH (0.07), which might be caused by different population composition, editing and/or modelling, or just because this was a subset data of our previous research (Miar et al. 2014b). When comparing the two paired measurements, the heritability estimate for a* on frozen-thawed samples was comparable to that on fresh samples

when their standard errors (S.E.) were considered. However, heritability estimates of L*, pH and drip loss were higher when using the data measured on fresh meat.

4.3.4. Correlations between and within meat quality characteristics of fresh and frozen-thawed pork

The phenotypic, genetic and environmental correlations of meat quality measurements between fresh and frozen-thawed pork are shown in Table 4.4. Measurements on fresh and frozen-thawed samples showed high (> 0.6) genetic correlations with the exception of b* (0.44) and drip loss (0.24). Moderate phenotypic correlations between fresh and frozen-thawed measurements were observed for L* and a*, while very low correlations were found for b* (0.06) and drip loss (0.14). The environmental correlations were moderate for all traits except for drip loss (0.07) (Table 4.4).

Furthermore, the genetic correlations estimated within fresh and within frozen-thawed samples (Table 4.5 and Table 4.6) showed that in fresh samples, L* and a* had low negative genetic correlation (-0.24) compared to medium negative correlation (-0.44) of its frozen-thawed counterpart. High genetic correlation (0.76) between L* and b* was observed in fresh samples and medium genetic correlation (0.48) was shown in frozen-thawed samples. Medium to high negative genetic correlation of L* and pH (-0.59) was shown in fresh samples compared with high negative correlation observed in frozen-thawed ones (-0.74). Genetic correlation between L* and drip loss was comparable within fresh samples (0.57) and frozen-thawed samples (0.51).

4.4. Discussion

4.4.1. Meat quality changes and disunity of measuring methods

Direct comparison of the data derived from drip loss and colour measurement conducted before (fresh) or after (frozen then thawed) freezing in this experiment were complicated by different methods being used to estimate drip loss and by the use of a different illuminant for measuring colour in fresh and frozen product. Colour data from the different illuminants were

compared in a paired t-test because CIE L*, a* and b* values between the illuminants are highly correlated ($r > 0.96$) and of little numerical difference (Sun et al. 2017), confirming that the color measurements from the fresh and frozen-thawed pork were comparable. Sun et al. (2017) concluded that the specific illuminant used (C or D65) was less important than its relationship with other measurements. Similarly, for pH, a pH meter standardized using commercially available standards would yield representative pH values regardless of its manufacturer.

The mechanism of this color change can be explained by the inter-convertibility of oxy-myoglobin (red), deoxy-myoglobin (purple) and met-myoglobin (brown). A related enzymatic reducing system which reduces met-myoglobin back to myoglobin may be responsible for the color change (Livingston and Brown 1981). The enzymatic reducing capacity in muscle is generally referred to as met-myoglobin reducing activity (MRA) (Abdallah et al. 1999) and in fresh muscles, due to high MRA, the originally formed brown met-myoglobin will be rapidly reduced to purple deoxy-myoglobin and then re-oxygenated to red oxy-myoglobin, making the meat "bloom". However, when the meat is frozen, met-myoglobin will accumulate on the surface of the meat due to decreased activity of the related enzymes and darken the color of the meat (Abdallah et al. 1999). The underlying mechanism is that the damage to mitochondria caused by ice crystals formed during freezing will result in the release of an enzyme β-hydroxyacyl CoA-dehydrogenase (HADH) from the mitochondrion into the sarcoplasm (Chen et al. 1988), leading to the depletion of nicotinamide adenine dinucleotide hydrogen (NADH), a co-factor essential for met-myoglobin reducing enzymes, resulting in the accumulation of met-myoglobin in the surface of the meat (Abdallah et al. 1984). At the same time, the related enzymes might be lost with the exudate caused by the thawing process, and/or due to oxidation during thawing, and/or

be used by other biochemical reactions (Leygonie et al. 2012a), and all these events may contribute to the loss of bloom.

In fresh meat, the intramuscular ultimate (24 h) pH is mainly affected by the muscle glycogen concentration at the time of slaughter (Bendall and Swatland 1988) and the rate of anaerobic glycolysis postmortem (Duclos et al. 2007). In frozen meat, anaerobic glycolysis, which contributes to the decline of muscle pH post-mortem, is restricted because of low temperature and the lack of available water. However, during the thawing process, anaerobic glycolysis will reinitiate if there is ATP remaining in the cell, causing a continuous decline of pH (Lundberg et al. 1987). Our results showed that muscle pH differed by 0.20 units from fresh to frozen-thawed samples, which is consistent with the aforementioned mechanism (Table 4.1). Alternatively, there is a possibility that exudate produced through thawing could denature buffer proteins, which in turn might lead to the release of hydrogen ions and the decline of meat pH (Leygonie et al. 2012a).

The differences in the methods used to assess drip loss in fresh and previously frozen product in this experiment, however, were substantial and there was clear confounding between method used and the treatment of the product. As a result, although drip loss before and after freezing and thawing was considered in genetic analysis, there were no paired t-tests between the fresh and frozen drip loss data. Freezing and thawing have been shown to contribute to decreased water-holding capacity of meat (Ngapo et al. 1999; Vieira et al. 2009). The cause of this water-holding capacity loss during freezing and thawing is believed to be related to the disruption of the muscle fibre spatial structure and the modification and denaturation of related proteins (Savage et al. 1990).The substantial difference in drip loss between the fresh and frozen-thawed pork most likely was not solely due to freezing and thawing in this study because two different

methods were used to assess drip loss, and so no statistical comparison was performed. The method employed prior to freezing for fresh measurement was based upon that described by Barton-Gade et al. (1994), while that used after freezing and thawing was based on that of Honikel (1998). Differences between the two methods worthy of note are that the Barton-Gade et al. (1994) method introduces contact with a grill for 48 h, whereas the Honikel-type method minimized contact by suspending the pork on a hook for 24 h. Further, the Barton-Gade et al. (1994) method trimmed only subcutaneous fat once the chop was deboned, whereas both subcutaneous fat and epimysium were trimmed in the Honikel (1998) method to standardize the sample size. The additional trimming applied in the Honikel (1998) method may have exacerbated drip loss as additional cutting would increase the cell damage incurred, although drip was allowed for only 24 h with the bag method rather than the 48 h of the horizontal grill method used for fresh. Both methods are gravimetric, however, and gravimetric methods tend to be highly correlated ($r = 0.90$ to 0.92) (Otto et al. 2006). Also, the difference in drip loss value expected from each of the two methods employed is about 2% (Enfält et al. 1997; Otto et al. 2006). As a consequence, examination of the efficacy of genetic selection for drip loss before or after freezing and thawing was warranted but interpreted with caution by taking into consideration that the drip loss methods were different.

4.4.2. The importance of fat content on meat quality

Fat occur in most tissues in the body and in four anatomical "depots", namely internal, subcutaneous, intermuscular and intramuscular (Webb 2003). Based on our meat consumption habit, intramuscular fat is primarily the portion being consumed among the four compartments, which makes it the most important fat factor influencing meat quality, especially for meat flavour, juiciness and tenderness. According to previous research, intramuscular fat influences meat flavour and juiciness directly and tenderness indirectly (Nishimura et al. 1999; Jeremiah et

al. 2003; Hocquette et al. 2010; Wood et al. 2008). Based on this, crude fat content may also have an effect on the changes of meat quality traits measured on fresh and frozen-thawed pork.

As shown in Figure 4.1, with the increase of crude fat content, the changes in L* (Figure 4.1 (A)) and b* (Figure 4.1 (C)) with freezing and thawing both increased and then decreased. As crude fat content increased up to 4% intramuscular fat, there was an associated increased difference in mean L* value between fresh and frozen-thawed pork (Figure 4.1 (A)). Why this occurred was unclear, although β-hydroxyacyl CoA-dehydrogenase activity that may reduce MRA may be increased in muscles with increased intramuscular fat because these muscles may have additional Type I oxidative muscle fibres, but muscle fibre type was not assessed in this study. Additionally, cell damage caused by freezing may compress and deform myofibrils (Grujić et al. 1993), which may increase light absorption, resulting in decreased L* value (Table 4.1). Because crude fat can reduce the muscle cell damage caused by freezing, it is expected that with the increased crude fat content, the difference of L* should be decreased as shown in Figure 4.1 (A). For pig skeletal muscles with more than 70% water (Dickerson and Widdowson 1960) the effective thermal conductivity will increase with decreasing temperature due to the freezing process; however, for muscles with increased fat content, the effective thermal conductivity will be relatively stable (Kumcuoglu et al. 2010). That is, the potential for cell damage caused by the ice crystals formed during freezing is greater in muscles with low intramuscular fat content than those with high fat content. The “turning point” in the difference between fresh and frozen-thawed L* values at about 3%~4% might be caused by the increased crude fat content itself leading to an increased reflection of light, counteracting the decreased light reflection caused by other mechanisms. For example, enzymes capable of reducing met-myoglobin to myoglobin might be lost with the exudate, contributing to the loss of bloom (decreased b*) (Livingston and

Brown 1981). The variation in the change in b* values (Figure 4.1 (C)), as well as in a* values (Figure 4.1 (B)),with freezing and thawing with crude fat content, can be explained in a similar manner, however, further additional mechanistic experiments on meat color and its relationship with crude fat content are needed to determine the causal relationships between the two.

4.4.3. Heritability and correlations

The heritability estimated on fresh meat pH was 0.07, which was within the range of 0.07 - 0.39 reported by other literature (Cameron 1990; Hovenier et al. 1992; De Vries et al. 1994; Hermesch et al. 2000; Suzuki et al. 2005; Van Wijk et al. 2005; Gjerlaug-Enger et al. 2010). However, the estimated heritability for pH on frozen-thawed samples was extremely low (0.02), indicating that the effects of freezing and thawing obscured genetic effects. This result indicated that pH will appear much less heritable in frozen-thawed meat than in fresh meat. The heritability of drip loss was moderate (0.33) for the fresh measurement, which agreed with other reported results that ranged from 0.14 to 0.33 (Hovenier et al. 1992; De Vries et al. 1994; Hermesch et al. 2000; Suzuki et al. 2005; Gjerlaug-Enger et al. 2010; Miar et al. 2014b), but was low for the frozen-thawed measurement (0.09). These results may have arisen from different methods being used for the fresh and frozen-thawed drip loss assessment; however, the methods used were both gravimetric methods and vertical and horizontal drip methods tend to be highly correlated to each other and to carcass characteristics (Otto et al. 2006). Again, the substantial influence of ice crystal damage would increase the random error variance related to drip loss (Ngapo et al. 1999; Vieira et al. 2009). In genetic evaluation and selection program, traits that are highly heritable will lead to increased accuracy of genetic prediction, and therefore will yield an increased genetic response to selection. For this reason, the use of fresh rather than frozen-thawed pork is recommended for pH and drip loss measurements for breeding and selection of pigs, with drip loss to be estimated using the Barton-Gade et al. (1994) method.

The genetic correlations as shown in Table 4.4 indicated that selection for L*, a* and pH on fresh pork was likely to cause genetic change in their corresponding frozen-thawed status. These results implied that genetic selection for L* and a* on frozen-thawed pork will concomitantly influence fresh pork appearance, which is reassuring as consumers frequently employ freezing and thawing procedures when managing personal food inventories. However, the relatively low genetic correlation of drip loss between fresh and frozen-thawed pork suggests that selection based upon the drip loss method of Honikel (1998) method using frozen-thawed pork would likely lead to decreased genetic improvement for fresh pork meat.

Water-holding capacity is very important for industrial carcass yield (den Hertog-Meischke et al. 1997; Huff-Lonergan and Lonergan 2005), and for consumers, it is closely related to the perceived value of a purchase (Offer and Trinick 1983; den Hertog-Meischke et al. 1997). However, due to the low genetic correlation between fresh drip loss measurements conducted using the Barton-Gade et al. (1994) method and frozen-thawed drip loss measurements with the Honikel (1998) method, instead of selecting for this trait on frozen-thawed pork directly, we should understand the freezing and thawing procedures that would ensure that drip loss variation due to freezing and thawing was minimized. As for meat color, greater genetic correlations were observed between fresh and frozen samples for L*, a*, and pH, indicating that prediction of frozen-thawed meat color from fresh meat color would be unreliable. Although the meat color may have no direct correlation with eating satisfaction (Carpenter et al. 2001), the favorable color of meat (red) will orient consumer purchasing because red color can stimulate appetite (Singh 2006), reinforcing the importance of developing other prediction methods for frozen-thawed meat color from fresh meat color.

In terms of environmental correlations, the estimated results were moderate for all traits except for drip loss (0.07), indicating that the same environments may determine the measurements of meat quality from fresh to frozen-thawed, that is, different freezing strategies, like time and temperature, will lead to the variation of meat quality changes from fresh to frozen-thawed status. In comparison, greater genetic correlations were observed between fresh and frozen samples for L*, a*, and pH, indicating that for these measurements, selection based on measures of frozen samples could lead to corresponding genetic progress in the fresh samples or vice versa. However, for b* and drip loss, genetic selection for fresh quality measurement based on measurement of frozen product or vice versa (with concomitant method considered with regard to drip loss) would lead to decreased genetic progress.

4.5. Conclusions

Based on the results that genetic correlations estimated in fresh and in frozen-thawed samples between L* and a*, L* and b*, a* and b* were all moderate to high, genetic correlations between a* and pH, b* and drip loss in frozen-thawed samples were low, as well as genetic correlations of pH and drip loss estimated in frozen-thawed samples were smaller than in fresh samples, it can be concluded that either fresh or frozen-thawed samples could be used for L*, a* and b* measurements, but pH and drip loss should be measured in fresh samples rather than in frozen-thawed ones for genetic selection.

Table 4.1. Paired t-test comparison of least squares means for meat quality measurements from fresh and frozen-thawed pork m. *longissimus dorsi*.

Traits	N	fresh pork (S.D.[a])	frozen-thawed pork(S.D.)	Mean difference ± S.E.M.[b]	*p* value of paired t-test
L*[c]	2021	48.49 (2.77)	44.46 (2.75)	-4.04 ± 0.08	<0.0001
a*[d]	2025	6.21 (1.59)	7.81 (1.21)	1.60 ± 0.03	<0.0001
b*[e]	2025	15.14 (2.00)	2.80 (1.20)	-12.34 ± 0.05	<0.0001
pH	2010	5.74 (0.18)	5.54 (0.15)	-0.20 ± 0.01	<0.0001

[a] Standard deviation

[b] Standard error of the mean

[c] Lightness

[d] Redness

[e] Yellowness

Table 4.2. Heritability estimates (± S.E.) and variance components for meat quality traits measured on fresh pork m. *longissimus dorsi*.

Measured traits	Fresh meat			
	Heritability	σ_e^{2} [a] ± S.E.[d]	σ_a^{2} [b] ± S.E.	σ_c^{2} [c] ± S.E.
L*	0.33 ± 0.05	2.97 ± 0.28	2.49 ± 0.42	2.12 ± 0.40
a*	0.25 ± 0.05	1.00 ± 0.08	0.58 ± 0.06	0.76 ± 0.13
b*	0.10 ± 0.03	1.12 ± 0.07	0.39 ± 0.09	2.19 ± 0.33
pH	0.07 ± 0.02	0.01 ± 0.00	0.00 ± 0.00	0.02 ± 0.00
Drip loss	0.33 ± 0.06	0.13 ± 0.01	0.08 ± 0.01	0.03 ± 0.01

[a] Residual variance

[b] Additive genetic variance

[c] Contemporary group effect

[d] Standard error

Table 4.3. Heritability estimates (± S.E.) and variance components for meat quality traits measured on frozen-thawed pork m. *longissimus dorsi*.

Measured traits	Frozen-thawed meat			
	Heritability	σ_e^{2}[a] ± S.E.[d]	σ_a^{2}[b] ± S.E.	σ_c^{2}[c] ± S.E.
L*	0.17 ± 0.04	3.21 ± 0.20	1.21 ± 0.26	2.78 ± 0.45
a*	0.32 ± 0.05	0.53 ± 0.05	0.44 ± 0.07	0.42 ± 0.07
b*	0.15 ± 0.03	0.56 ± 0.03	0.19 ± 0.04	0.55 ± 0.09
pH	0.02 ± 0.02	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Drip loss	0.09 ± 0.03	4.70 ± 0.23	0.64 ± 0.23	1.59 ± 0.29

Table 4.4. Phenotypic, genetic and environmental correlations and their standard errors between meat quality traits measured on fresh and frozen-thawed pork m. *longissimus dorsi*.

Traits (between fresh and frozen)	Phenotypic corr.	Genetic corr.	Environmental corr.
L*	0.24 ± 0.04	0.67 ± 0.09	0.35 ± 0.05
a*	0.42 ± 0.04	0.82 ± 0.06	0.33 ± 0.05
b*	0.06 ± 0.06	0.44 ± 0.14	0.34 ± 0.04
pH	0.18 ± 0.05	0.85 ± 0.54	0.26 ± 0.03
Drip loss	0.14 ± 0.03	0.24 ± 0.19	0.07 ± 0.04

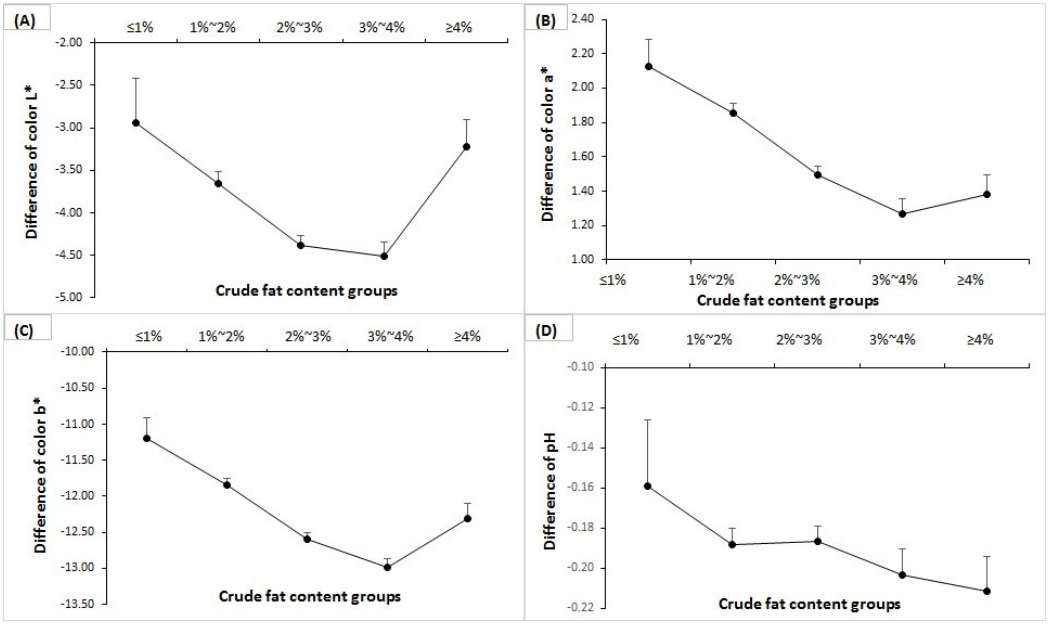
Table 4.5. Phenotypic correlations (above diagonal) and genetic correlations (below diagonal) and their standard errors for meat quality traits measured on fresh pork m. *longissimus dorsi*.

Trait	L*	a*	b*	pH	Drip loss
L*		0.36 ± 0.04	0.76 ± 0.02	-0.26 ± 0.05	0.39 ± 0.03
a*	-0.24 ± 0.13		0.79 ± 0.02	-0.16 ± 0.05	0.35 ± 0.03
b*	0.76 ± 0.06	0.36 ± 0.13		-0.18 ± 0.07	0.39 ± 0.03
pH	-0.59 ± 0.11	-0.35 ± 0.14	-0.61 ± 0.12		-0.19 ± 0.04
Drip loss	0.57 ± 0.09	0.16 ± 0.13	0.66 ± 0.10	-0.62 ± 0.12	

Table 4.6. Phenotypic correlations (above diagonal) and genetic correlations (below diagonal) and their standard errors for meat quality traits measured on frozen-thawed pork m. *longissimus dorsi*.

Trait	L*	a*	b*	pH	Drip loss
L*		-0.42 ± 0.03	0.58 ± 0.03	-0.12 ± 0.05	0.06 ± 0.04
a*	-0.44 ± 0.11		0.10 ± 0.05	-0.05 ± 0.04	-0.05 ± 0.04
b*	0.48 ± 0.11	0.45 ± 0.11		-0.19 ± 0.05	0.02 ± 0.04
pH	-0.74 ± 0.09	0.08 ± 0.28	-0.97 ± 0.42		-0.10 ± 0.04
Drip loss	0.51 ± 0.18	-0.33 ± 0.16	0.13 ± 0.19	-0.46 ± 0.35	

Figure 4.1 (A, B, C, D). Changes of meat quality from fresh to frozen-thawed with crude fat content. Crude fat content is expressed as percentage of wet tissue and divided into five groups: ≤ 1%, 1%~2%, 2%~3%, 3%~4% and ≥ 4%.



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5. Genome-wide association study of collagen in beef cattle

5.1. Introduction

Among all meat quality attributes, anticipated tenderness is one of the most important factors with regard to eating satisfaction considered by consumers when purchasing and re-purchasing meat, and it is determined indirectly by meat color, visible fat and type of cut at the point of purchase (Robbins et al. 2003). Meat toughness, commonly measured by Warner-Bratzler Shear Force (WBSF), is a complicated index of myofibrillar degradation, sarcomere length, proteolytic activity and insolubility of collagen (Bongiorni et al. 2016).

The major factor responsible for cooked meat background toughness is intramuscular connective tissue (IMCT), which contains fibers of the proteins collagen and elastin surrounded by a proteoglycan (PG) matrix (Purslow 2005). IMCT has three levels of structure, specifically the endomysium, perimysium and epimysium, with the epimysium surrounding the whole muscle and is not considered to affect meat quality because it is usually trimmed off the meat at consumption following cooking (Purslow 2014). Collagen, composing 1-10% of the dry mass of skeletal muscle (Bendall 1967), is the main protein of IMCT and it influences meat toughness through the strength and density of its intermolecular crosslinks. What is worthy of note is that IMCT is a dynamically remodeled structure that is degraded and expanded with the hypertrophy of muscle fibers that occurs during the growth of animals, during which immature divalent cross-links of collagen are formed. However, with the growth of muscles, the immature divalent cross-links will spontaneously form into mature trivalent crosslinks from allysines or hydroxyallysines in close proximity to each other, which will make the meat additionally heat resistant and hence of increased toughness (Purslow 2014).

To date, only two types of trivalent intramuscular collagen cross-links have been identified, specifically pyridinoline (PYR) and Ehrlich's Chromogen (EC), and it has been

shown that increased PYR and EC concentrations are associated with increased collagen heat stability (Horgan et al. 1990), which could contribute to increased meat toughness. Recently, Taye et al. (2017) reported that the gene *COL9A2* (collagen type IX alpha 2 chain) may have some effects on muscle structure through the modification of collagen and hence affect meat tenderness in Ankole cattle (Taye et al. 2017), and their results agreed with those of Chang (2007) and Ghosh et al. (2015). In chicken, RNA-Seq analysis using broiler chickens detected six genes (*P4HA3*, *LEPREL4*, *PCOLCE2*, *COL16A1*, *COL20A1* and *VWA1*) were differentially expressed and may be associated with collagen synthesis related to WBSF (Piórkowska et al. 2016). The studies mentioned above did not, however, actually substantiate the phenotypic contribution of intramuscular collagen content or solubility to meat toughness.

The objectives of the current study were, therefore, to identify variations (SNPs) in genes along the genome associated with total collagen and collagen solubility in the bovine m. gluteus medius, and to explore the biological relevance of the genes to beef toughness.

5.2. Materials and methods

All animals used in this study were managed according to the guidelines of Canadian Council on Animal Care (CCAC 1993) and the experimental procedures were approved by an ethics committee at the University of Alberta (AUP00000777).

5.2.1. Animals and management

In total, three beef cattle breeds raised and managed at Roy Berg Kinsella Ranch, University of Alberta, Canada, were used in the present study, and they were Angus purebred (n = 45), Charolais purebred (n = 45) and Kinsella Composite (n = 47). The Kinsella Composite is a cross breed between Angus, Charolais, or Alberta Hybrid bulls and the University of Alberta's hybrid dam line generated by crossing composite cattle lines of multiple beef cattle breeds (Goonewardene et al. 2003). All animals used in the present study were born between March to

May of 2013 and 2014, and they were weaned by about six months of age. After that, the animals were fed with a background diet of 80% barley silage, 17% barley grain, and 3% rumensin pellet supplement. For the finishing diet, 75% barley grain, 20% barley silage, and 5% rumensin pellet supplement was used. The detailed information about these beef cattle breeds and their management can be found elsewhere (Nkrumah et al. 2007; Mao et al. 2013; Mukiibi et al. 2018).

5.2.2. Phenotypic measurement

5.2.2.1. Extraction of soluble and insoluble collagen in freeze dried meat

After slaughter, the left gluteus medius muscles were removed from each carcass, cut into 2.5 cm thick steaks and two steaks were aged for either three days (3 day postmortem, 3 dpm) or 13 days (13 day postmortem, 13 dpm) at 4 °C. The steak aged for 3 dpm was thawed, trimmed of external fat and epimysium, chopped into 2 mm^2 cubes, which were mixed by hand and then divided into two portions. Each portion was weighed, frozen at -20 °C, and then one of the portions was lyophilized and stored in -20 °C until use. The steak aged for 13 dpm was treated similarly, with the exception that the whole steak was trimmed, chopped, weighed, frozen and lyophilized. To extract soluble collagen for further assay, the Hill (1966) method was applied with some modifications. Briefly, about 2 g of freeze dried meat were weighed and homogenized with 20 mL of Quarter-strength Ringer's solution and incubated in 77 °C water bath for 63 minutes. After incubation, the solution was centrifuged at 3500 g for 10 minutes and the supernatant was collected for the quantification of soluble collagen content. Total residue inside the tubes was weighed to quantify the insoluble collagen fraction.

5.2.2.2. Hydroxyproline assay for soluble and insoluble collagen

The extracted soluble and insoluble collagen fractions from 5.2.2.1 were used to determine hydroxyproline content through the method of Bergman and Loxley (1963) with some

modifications. Briefly, 1 mL of the extracted soluble collagen fraction was hydrolyzed in a capped glass test tube with 4 mL of 6 M and 1 mL of 12 M HCl. The tubes were carefully purged with nitrogen gas for approximately 10 seconds and capped properly followed by the hydrolyzation process for 20 hours. For the quantification of insoluble collagen fraction, about 0.3 g of wet residue from 5.2.2.1 was taken and hydrolyzed with 5 mL of 6 M HCl. After hydrolysis (20 hours), the hydrolysate was cooled with ice water for several minutes and filtered with No. 4 filter paper. After filtering, the solution was evaporated to dryness and reconstituted with 2 mL deionized (DI) water and neutralized with NaOH. Finally, the neutralized fraction was evaporated again to dryness and reconstituted with DI water to a constant volume of 5 mL.

For the quantification of hydroxyproline, 1 mL of reconstituted sample solution from above was taken and pipetted into a glass tube, followed by the addition of 2.0 mL isopropanol and well mixed with a vortex. Then, 1.0 mL of oxidant solution (mixture of 7% (w/v) chlororamine T and acetate/citrate buffer (0.42 M sodium acetate, 0.13 M trisodium citrate, 0.03 M citric acid and 38.5% isopropanol), at a ratio of 1:4 (v/v)) was added into the tube and mixed thoroughly, followed by the incubation at room temperature for 4 ± 1 minutes. After incubation, 13 mL of Ehrlich's reagent solution (2 g of p-dimethylaminobenzaldehyde in 3 mL of 60 % (v/v) perchloric acid (w/v) and isopropanol at a ratio of 3:13 (v/v)] was added into the tube and immediately the tube was capped, mixed with a vortex and incubated at 60 °C for 25 minutes and then cooled down in ice water for about 10 minutes. Finally, the cooled solution was transferred into a graduated flask and made up to 50 mL with isopropanol. The water blanks were prepared in the same manner by using deionized water instead of sample solutions. The standards were made through gradient dilution (2.5, 5.0, 10.0, 20.0 and 40.0 μg/mL) of a stock solution of trans-4-hydroxy-L-proline (Sigma-Aldrich Canada Ltd, Oakville, ON). The

absorbance of the sample solution was obtained at 558 nm against a water blank (Genesys 20 Spectrophotometer, Thermo Scientific, Canada). The hydroxyproline concentration was derived from the regression of expected trans-4-hydroxy-L-proline standard concentrations and their absorbances at 558 nm. A conversion factor of 7.14 was used to convert hydroxyproline content to collagen content according to Stanton and Light (1987). For the collagen content measurement, duplication was performed and the average was used for analysis.

5.2.2.3. Total collagen and collagen solubility

Total collagen content was calculated by adding soluble and insoluble collagen fractions together. Collagen solubility (%) was calculated by dividing soluble collagen content (mg/g raw meat) with total collagen content (mg/g raw meat) and multiplying by 100.

5.2.3. Genotyping and quality control

Blood samples from all studied animals were collected and genotyping was performed using the Illumina Bovine SNP50_v2 Beadchip, which features 54609 SNPs in total. The SNP position was extracted from the UMD 3.1 bovine assembly and used for the analysis. All the SNPs with minor allele frequency (MAF) less than 5% or SNP missing rate higher than 10% were excluded from the dataset. After quality control, there were 43523 SNPs for genome-wide association analysis.

5.2.4 Genome-wide association study (GWAS)

Before GWAS, all phenotypic records (total collagen content and collagen solubility) outside the mean ± 3 standard deviations (SD) were considered as outliers and removed from the data set before use.

Possible factors, including breed (Angus, Charolais and Kinsella Composite), sex, pen, slaughter year, slaughter batch, days between birth to slaughter, and hot carcass weight were tested using a generalized linear model with R/stats (R Core Team, 2013) by comparing the full

model with reduced models, and the significant factors (breed, and slaughter batch) were included in the GWAS model.

The estimation of variance components and GWAS were performed by using single-step Genomic Best Linear Unbiased Prediction (ssGBLUP), which combines all phenotype, pedigree and genotype information in one step (Misztal et al. 2009; Christensen and Lund 2010; Wang et al. 2012). According to Wang et al. (2014), ssGBLUP calculates SNP effects using an iterative process, which increases the weight of SNPs with relatively larger effects and reduces the weight of SNPs with smaller effects. For GWAS, the BLUPF90 family programs (Misztal et al. 2002; Aguilar et al. 2011) were used and the animal model was as follows:

$$\mathbf{Y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$$

where **Y** is the vector of phenotypic records (total collagen content and collagen solubility); **b** is the vector of fixed effects; **a** is the random additive genetic effects with [u ~ N (0, $\mathbf{H}\sigma_a^2$], where **H** is the relationship matrix combining A (numerator relationship matrix) and G (genomic matrix) matrices, and σ_a^2 is the polygenetic additive variance; **e** is the vector of residual errors with a distribution of [**e** ~ N (0, $\mathbf{I}\sigma_e^2$)], where **I** is the identity matrix and σ_e^2 is the residual variance. **X**, and **Z** are the incidence matrices for **b**, and **a**. In this model, beef cattle breed (Angus, Charolais and Kinsella Composite) and slaughter batch (seven levels) were included as fixed effects. The *prior* distribution of additive genetic, slaughter batch and residual variance components was the inverted Wishart and POSTGSF90 program was used to generate the posterior estimates (Aguilar et al. 2011).

The inverse **H** matrix can be described as follows:

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{G}^{-1} - \mathbf{A}_{22}^{-1} \end{bmatrix}$$

where $\mathbf{A}_{22}^{-1}$ is the inverse relationship matrix generated using animal pedigree information (In total, 1,026 animals were in the pedigree file and there were 319 sires available) and $\mathbf{G}^{-1}$ is the inverse genomic relationship matrix generated using an iterative procedure by weighting each SNP effect through its expected variance as described by VanRaden (VanRaden 2008). The calculation of G can be written as:

$$\mathbf{G} = \mathbf{ZDZ'q}$$

where **Z** is the marker incidence matrix with a genotype format as 0/1/2 and it is adjusted for allele frequency before use; **D** is the diagonal matrix with the inverse of the expected SNP variance; **q** is the weighting factor (equal to zero for the first time) which is used to make sure the average diagonal in **G** matrix is close to $\mathbf{A}_{22}$ matrix (Vitezica et al. 2011). Briefly, the analyzing steps of ssGBLUP method were as follows: the D matrix was initialized through **D** = **I** first; second, **G** matrix was calculated using the formula **G** = **ZDZ´q**; third, the genomic estimated breeding value (GEBV) was calculated; fourth, GEBV was converted to SNP effects using the equation of $\hat{u} = \frac{\sigma_u^2}{\sigma_a^2}\mathbf{DZ'G}^{*-1}\hat{a}_g = \mathbf{DZ'}\ [\mathbf{ZDZ'}]^{-1}\hat{a}_g$ (Wang et al. 2012), where $\hat{u}$ is the vector of SNP marker effect, $\hat{a}_g$ is the GEBV of the genotyped animals; fifth, the weight for each SNP marker was calculated using the equation $d_i = \hat{u}_i^2 2p_i(1\text{-}p_i)$, where *i* is the *i*th SNP marker; and sixth, the SNP weight was normalized to make it remain constant with the total genetic variance and the second step repeated.

The final results from the above GWAS were reported as the proportion of genetic variance explained by a consecutive sliding window of 10 adjacent SNPs, and SNPs windows that explained more than 1% of the total proportion of genetic variance were treated as significant. SNP windows were used instead of only using a single SNP because a QTL is

usually surrounded by many SNPs that are in linkage disequilibrium with the QTL (Habier et al. 2011) and including those can provide additional power compared to using a single SNP and capture the possible QTL effect of the traits of interest. Also, by using SNP windows, statistical noise can be reduced (Sun et al. 2011).

5.2.5. Functional enrichment analysis

The SNP windows (10 SNPs per window) that explained more than 1% of the total proportion of genetic variance from above GWAS were used to search for nearby (within 500 Kb upstream and 500 Kb downstream of the SNPs) RefSeq genes through Ensembl BioMart (Zerbino et al., 2017) (release 92). Ingenuity Pathway Analysis (IPA) was used to visualize and explore the biological mechanisms, pathways and functions of these identified genes (http://www.ingenuity.com). The "Core Analysis' function included in IPA was selected to interpret the data and the probability of each assigned gene function being due to chance alone was tested using the Fisher Exact test with significance at $p < 0.05$.

5.3. Results

5.3.1. Total collagen and collagen solubility

The least squares means (standard error) of total collagen and collagen solubility for both 3 days postmortem (dpm) and 13 dpm are shown in Table 5.1. There was significant difference ($p < 0.05$) between total collagen and collagen heat solubility values at 3 and 13 dpm (Table 5.1).

5.3.2. Genome-wide association study

In total, 7 SNP windows that explained more than 1% of additive genetic variance were detected for 3 dpm total collagen content (Table 5.2), and 9 SNP windows were detected for 3 dpm collagen solubility (Table 5.3). For 13 dpm, 10 and 6 SNP windows were detected for total collagen (Table 5.4) and collagen solubility (Table 5.5), respectively. Manhattan plots for total collagen and collagen solubility are shown in Figure 5.1 to 5.4 below. Although there are some

significant windows detected within the same chromosome for the four measured traits (e.g.: chromosome 7 for 3 dpm total collagen, 13 dpm total collagen and collagen solubility; chromosome 8 for 3 dpm total collagen and 13 dpm total collagen; chromosome 12 for 3 dpm total collagen and collagen solubility; chromosome 19 for 3 dpm total collagen and collagen solubility, 13 dpm total collagen), no overlapping significant windows were found (Table 5.2 to 5.5). Detailed SNP information within the significant windows for total collagen and collagen solubility are shown in Supplementary Table 5.1 to 5.4.

5.3.3. Functional analysis

In total, 64 Ensemble RefSeq genes were detected harboring the SNPs windows (within 500 Kb upstream and 500 Kb downstream) that explained more than 1% of additive genetic variance for 3 dpm total collagen content (Supplementary Table 5.5). For 3 dpm collagen solubility, 128 candidate genes were detected (Supplementary Table 5.6), while for 13 dpm total collagen and collagen solubility, 144 (Supplementary Table 5.7) and 36 (Supplementary Table 5.8) genes were found, respectively. Genes detected using RefSeq were related to nervous system development and function, cell-mediated immune response, and connective tissue development and function among others (Figure 5.5 to Figure 5.8). The genes under the category of Connective Tissue Development and Function may be related to collagen and were summarized in Table 5.6 to 5.9.

5.4. Discussion

5.4.1. Importance of total collagen and collagen solubility on meat toughness

Great attention has been paid to meat tenderness by breeders and researchers as it is one of the most important factors considered by consumers when purchasing red meat (Robbins et al. 2003). To date, there are mainly three components contributing to meat tenderness, namely myofibrillar proteins, intramuscular fat and connective tissue (Van Laack et al. 2001). Based on

early studies (Bouton et al. 1975; Møller 1981), WBSF deformation curves showed that commonly used treatments, such as aging and cooking, can only influence muscle fibers (myofibrillar proteins). However, meat toughness caused by connective tissue cannot be reduced substantially by any treatment from the point of animal slaughter to the point of cooking the meat, which initiated the name of *background toughness* (Marsh and Leet 1966). There is controversy about the correlation of total collagen content and meat tenderness. According to Reagen et al. (1976), an animal's age and total collagen content of the muscles contribute most of the variability of the tenderness in beef. However, Davis et al. (1979) concluded that, for US Choice-A maturity beef, the amount of collagen is not important in terms of meat tenderness and according to Wilson et al. (1954), the total collagen content of the longissimus dorsi muscle of veal was greater than that of steers or cows, suggesting that intramuscular total collagen does not adequately explain the variation of meat toughness caused by connective tissue (Hill 1966). With those results in mind, we suggest that the importance of total collagen content to meat tenderness should be explained with the interaction of animal age, different skeletal muscles (e.g. longissimus thoracis et lumborum, gluteus medius,), as well as the sample location within a muscle. In the present study, the total collagen content decreased significantly from 3 dpm to 13 dpm (Table 5.1), which does not agree with other reports (Palka 2003; Li et al. 2008) that indicated that total collagen content was relatively stable during aging. This is most likely due to an anatomical effect, because the steaks aged 3 and 13 dpm, although side by side in the muscle, were taken from different portions of the gluteus medius muscle for the quantification of total collagen. Given the small size of this muscle, a 2.5 cm thickness would be proportionally greater than the same distance in a muscle larger than the gluteus medius such as the semimembranosus

or longissimus thoracis et lumborum, suggesting that location effects may have influenced the total collagen content observed at each day postmortem.

This positional influence most likely also explains how collagen solubility decreased rather than increased with additional days of ageing. Collagen solubility of bovine muscle is expected to increase with time post mortem (Palka 2003; Li et al. 2008) although Jeremiah and Martin (1981) found no differences in bovine total collagen content or proportion of soluble collagen with post mortem ageing. Pierson and Fox (1976) also found no difference in the salt or acid solubility of bovine collagen with post mortem ageing. That collagen solubility decreased rather than increased with ageing suggested a decrease in the heat solubility of the collagen with position as the steak used for 13 dpm measurements was located close to the end of the muscle where intramuscular tendon may be located. Intramuscular tendon may have reduced heat solubility, but this hypothesis was not further investigated.

5.4.2. Single step GWAS

In the scientific literature there are several GWAS studies that have been done to detect SNPs that were associated with beef meat quality using single marker association strategy (Gill et al. 2009; Pannier et al. 2010; Sasago et al. 2017). However, due to the existence of linkage disequilibrium, relatively high rate of type I errors were observed along with the single marker association analysis (Vallejo et al. 2017). Recently, a method called single-step GWAS (ssGWAS) was developed and has been widely used to identify significant SNPs associated with complex quantitative traits in animal genetic studies (Misztal et al. 2013; Lemos et al. 2016; Magalhães et al. 2016; Carvalho et al. 2017; Wu et al. 2018) because of the advantage of its relatively high statistical power and prediction precision (Wang et al. 2012). One of the advantages of using ssGWAS is that it can combine both pedigree and genotype information of

the animals to gain more accurate estimation of the breeding values (Wang et al. 2012). Another advantage of using ssGWAS is that it can reduce the effects of SNPs with minor effect close to zero through iteration (Wu et al. 2018). Based on previous research, two iterations should be considered in order to gain the best accuracy of the estimated SNP effects while doing GWAS (Wang et al. 2012; Melo 2015). In the present study, the weighted SNP marker was generated in the first iteration and then used in the second round to calculate the SNP effect. By using the genomic information twice, there is a possibility of over estimating of the SNP effects, so the results should be interpreted with caution.

5.4.3. Candidate genes for meat toughness caused by intramuscular connective tissue

Despite an extensive review of the literature, few GWAS studies related to total collagen and collagen solubility in raw red meat were located. Ramayo-Caldas et al. (2016) conducted GWAS on 17 muscle conformation-related traits, among which insoluble collagen content was included. To date, this is the only GWAS study to the author's knowledge presenting significant SNPs that are associated with collagen characteristics and it addresses collagen insolubility only. The present study, therefore, has served to identify new genes that may be worthy of further validation in terms of their relationship with collagen solubility and total content. Based on the IPA results, 5 genes (Table 5.6: *PCSK5*, *SIN3B*, *CDC42SE2*, *PLVAP*, *F2RL3*) were related to 3 dpm total collagen, 18 genes (Table 5.7: *CCL3*, *CCL4*, *CCL5*, *BAIAP2*, *SLFN14*, *PCSK5*, *ARHGDIA*, *GFI1B*, *MAFG*, *GCGR*, *SIRT7*, *ASPSCR1*, *C1QL4*, *MCRS1*, *RAC3*, *RALGDS*, *RPTOR*, *TSC1*) were associated with 3 dpm collagen solubility, 19 genes (Table 5.8: *CD244*, *CD48*, *MSX2*, *RASD1*, *SREBF1*, *E2F2*, *ID3*, *ENC1*, *KDM1A*, *MAP2K3*, *STAT1*, *TNFRSF13B*, *C3*, *FCER1G*, *TNFSF14*, *EPHB2*, *HEXB*, *LLGL1*, *RAI1*) were related to 13 dpm total collagen, and 3 genes (Table 5.9: *DYSF*, *HSD11B1*, *LAMB3*) were related to 13 dpm collagen solubility.

These genes were involved in connective tissue development and function, and may be treated as candidate genes for meat toughness caused by IMCT.

Of the genes relating to 3 dpm total collagen, the *PCSK5* (*Proprotein Convertase Subtilisin/Kexin Type 5*) gene is a protein coding gene that encodes a member of the subtilisin-like proprotein convertase family, which is a class of enzymes that cleave proproteins (Seidah and Chretien 1999). According to Bauersachs, et al. (2005), the relative gene expression of *PCSK5* was up regulated at oestrus compared to dioestrus in the bovine and it was involved in the cleavage of IGF-1. By stimulating fibroblast replication and/or collagen synthesis, IGF-1 can increase collagen deposition (Butt et al. 1995). Further study is needed to validate if *PCSK5* is associated with total collagen.

SIN3B (*SIN3 transcription regulator family member B*) is a protein coding gene acting as a transcriptional repressor, and it was first identified as a repressor of the yeast *HO* gene in *Saccharomyces cerevisiae* (Sternberg et al. 1987). *SIN3B* is one of the eight subunits of the Sin3 core complex (associate with other regulatory proteins to control gene expression through deacetylation of nucleosomes) in mammalian cells (Silverstein and Ekwall 2005). In the mouse, Sin3B mediated the onset of cell quiescence through transcriptional repression of E2F-target genes, suggesting its role of preventing tumorigenesis (Grandinetti and David 2008). In addition, Grandinetti et al. (2009) investigated the role of SIN3B-null fibroblasts on senescence and showed that SIN3B is essential for the induction of senescence. Collaborating with other repressors (regulatory factor for X-box 5, histone deacetylase 2, G9a (a histone H3K9 methyltransferase), Sin3B is responsible for pro-inflammatory cytokine interferon gamma induced collagen type I gene (*COL1A2*) repression in vascular smooth muscle cells (Weng et al. 2014). According to Van Oevelen et al. (2010), Sin3A and Sin3B proteins were associated with

the maintenance of the differentiated skeletal muscle cells, suggesting their possible role in intramuscular connective tissue development.

The *PLVAP* gene codes for the plasmalemma vesicle associated protein, and being a single-span, type II membrane N-glycosylated glycoprotein (Stan et al. 2004), *PLVAP* is an endothelial cell-specific protein expressed in capillaries and veins throughout the body and thus plays a key role in angiogenesis and vascular permeability (Wiśniewska-Kruk 2014), suggesting its possible role in collagen synthesis. Further studies are needed to investigate if the *PLVAP* gene is involved in connective tissue development.

The *F2RL3* (*F2R like thrombin or trypsin receptor 3)* gene, located on chromosome 19, is expressed in different cell types and serves as a genetic marker of cardiovascular disease (Vergnolle et al. 2002; Hossain et al. 2015). A cell surface protein PAR-4 (thrombin protease-activated receptor 4) is encoded by *F2RL3* gene and activation of PAR-4 is essential for the regulation of vascular endothelial cell activity (Kataoka et al. 2003), suggesting its possible role in vascular development.

Of the genes associated with 3 dpm collagen solubility, *CCL3*, *CCL4*, and *CCL5* (*C-C Motif Chemokine Ligand* 3, 4, and 5) are all protein coding genes, and they belong to cytokine genes clustered on the q-arm of chromosome 17. Being one of the osteoclast-associated chemokines, over expression of CCL3 and enhanced activation of matrix metalloproteinases 9 (MMP-9) can increase osteoclast differentiation by TNF-like protein 1A (Collins et al. 2017), suggesting the possible collaboration of CCL3 and MMP-9, and MMP-9 is responsible for collagen (type IV) degradation (Roach et al. 2002). The *BAIAP2* (*BAI1 associated protein 2*) gene encodes for a brain-specific angiogenesis inhibitor (BAI1)-binding protein BAIAP2 active mainly in neurons (Oda et al. 1999). Also know as IRSp53 (insulin receptor substrate p53),

BAIAP2 is tyrosine phosphorylated by the insulin receptor and IGF-1 (insulin-like growth factor 1) receptor (Yeh et al. 1996), and serves as an important regulator of membrane and actin dynamics (Ahmed et al. 2010; Suetsugu et al. 2010; Kang et al. 2016), indicating its possible role in collagen synthesis and connective tissue development.

The *GFI1B* (*growth factor independent 1B transcriptional repressor*) gene, located on chromosome 9, is a protein coding gene which encodes a zinc-finger containing a transcriptional regulator that is primarily expressed in cells of hematopoietic lineage and is necessary for development and differentiation of erythroid and megakaryocytic lineages (Elmaagacli et al. 2007). This 37 kDa nuclear protein can silence transcription by binding to specific target gene promoters or γ -satellite sequences (Vassen et al. 2006). Mutations in *COL6A1*, *COL6A2* and *COL6A3* genes can cause Collagen VI myopathies like Ullrich congenital muscular dystrophy and Bethlem myopathy, and by using deep RNA profiling, Scotton et al. (2016) found that *GFI1B* gene was differentially expressed in collagen-VI-null mice, suggesting its possible role in Collagen VI myopathies. The *MAFG* (*MAF BZIP transcription factor G*) gene encodes for MAF BZIP Transcription Factor G, which is one of the small Maf proteins (MafF, MafG and Mafk), and MafG has a characteristic basic region linked to a leucine zipper domain which mediates DNA binding (Kataoka et al. 1996; Motohashi et al. 1997). Fibrosarcoma is a disease associated with the *MAFG* gene, and occurs in fibrous connective tissue (Howell and Burkes Jr 1977; de Aguiar Vallim et al. 2015), suggesting that the *MAFG* gene may be involved in the connective tissue disorder. The gene *SIRT7* (*Sirtuin 7*) is a protein coding gene that encodes a member of the sirtuin family of proteins and, being one of the seven different homologs of yeast Sir2, Sirt7 is localized mainly in the nucleoli and regulated RNA polymerase 1 transcription (Ford et al. 2006). Araki et al. (2015) showed that *SIRT7* knock out mice have reduced fibrosis, fibroblast

differentiation, and concluded that Sirt7 can maintain transforming growth factor receptor 1 through modulating autophagy and is essential for tissue repair process. *SIRT7* also serves as an important regulator of cartilage homeostasis through suppressing the transcriptional activity of *SOX7* (*Sry-type HMG box*), which is a transcription factor for chondrogenesis and responsible for cartilage maintenance by regulating the expression of cartilage-specific genes like *COL2A1* (*collagen type 2 alpha 1*) (Henry et al. 2012; Korogi et al. 2018). The *RPTOR* (*regulatory associated protein of MTOR complex 1*) gene, located on chromosome 17, is a protein coding gene encodes a component of a signaling pathway that regulates cell growth in response to nutrient and insulin levels, and it is involved in the control of the mammalian target of rapamycin complex 1 (mTORC1) activity which regulates cell growth and survival (Hara 2002). Serving as an essential scaffolding protein of the mTOR complex 1 (mTORC1), Raptor is able to promote mTORC1 activity via phosphorylation of its proline-directed residues, Ser8, Ser696, and Ser863 (Carriere et al. 2011). According to Selvarajah et al. (2019), mTORC1 can promote collagen biosynthesis through amplification of the activating the transcription factor 4-dependent de novo serine-glycine pathway. Hence, the *PRTOR* gene may play a role in the collagen synthesis and connective tissue development.

For the genes related to 13 dpm total collagen, the *EPHB2* (*EPH Receptor B2*) gene encodes a member of the Eph receptor family of receptor tyrosine kinase transmembrane glycoproteins (GeneCards). Popov et al. (2017) suggested the downregulation of *EPHB2* may contribute to inferior or delayed tendon healing, which is very common in aged people, suggesting its role in connective tissue remodeling. According to Du et al. (2017), *EPHB2* was differentially expressed between slow-growing and fast-growing broilers, suggesting a possible role in the regulation of skeletal muscle development. The *MSX2* (*Msh Homeobox 2*) gene

encodes a member of the muscle segment homeobox gene family, and there are two isoforms of human *MSX* genes (*MSX1* and *MSX2*) (Hewitt et al. 1991; Jabs et al. 1993). Cbfa1 is a Runt-related osteoblast-specific transcription factor involved in osteoblast differentiation and it regulates the osteoblast-specific expression of type I collagen genes (*COL1A1* and *COL1A2*) by binding to the genes' consensus Cbfa1-binding sites, located in the promoter region of the genes (Kern et al. 2001). MSX2 protein can also bind to the binding site of *COL1A1* and repress the expression of the gene (Dodig et al. 1996). Research showed that *MSX2* is upstream from *Cbfa1* (Satokata et al. 2000), suggesting its role in the regulation in type 1 collagen tynthesis. The *SREBF1* (*sterol regulatory element binding transcription factor 1*) gene, located on bovine chromosome 19, is a key transcription factor in adipocytes and acts as a candidate gene of lipogenic capacity through regulating the expression of lipogenic related genes (Zhang et al. 2003). Previous research showed that an 84-bp indel in the intron 5 of the *SREBF1* gene was associated with monounsaturated fatty acids content in Japanese Black cattle (Hoashi et al. 2007), and more recently, Lee et al. (2013) identified six polymorphic SNPs and validated 84-bp indel variation of *SREBF1* gene in Korean commercial cattle that were associated with fatty acid composition and marbling score. The *ID3* (*inhibitor of DNA binding 3*) gene encodes a helix-loop-helix (HLH) protein that heterodimerizes with HLH transcription factors to inhibit DNA binding of HLH proteins (Espira et al. 2009). By interacting with the protein named scleraxi, the *ID3* gene was able to inhibit the production of scar-associated collagen type 1α2 and 3α1 and hence slow the skin wound repair process (Teo et al. 2017). To examine the role of Id1 and Id3 in bone metabolism, Maeda et al. (2004) compared wild type mice and Id1/Id3 heterozygous knock out mice and found out that Id1/Id3 can promote bone formation in vivo. Futher studies are needed to investigate the function of *ID3* gene in the metabolism of muscle connective tissue.

The *C3* (*complement C3*) gene encodes for complement C3, which is the most common complement protein in human serum and it was originally identified as an anaphylatoxin (Stadelmann et al. 1998). To investigate the role of complement C3 in wound healing, Sinno et al. (2013) found that fibronectin and collagen I contents were increased in C3 treated wounds, suggesting that C3 was able to increase collagen deposion and promote wound healing. According to Shields et al. (2011), C3 and C4 can bind to collagen and elastin fibers within the adventitia through covalent thiolester bonds, leading to increased vascular stiffness, suggesting a cooperative relationship between C3, C4 and collagen. However, there is no research on C3 in skeletal muscle and so its possible role in connective tissue development needs to be elucidated. The *TNFSF14* (*TNF Superfamily Member 14*) gene, known as *LIGHT*, is a member of the TNF super family and the protein LIGHT is a key component of the TNF-lymphotoxin network (Steinberg et al. 2011; Ware and Šedý 2011). In humans, high levels of LIGHT were associated with bone loss (Brunetti et al. 2014), suggesting its role in maintaining bone, and *LIGHT*-deficient mice showed spine deformity and reduced femoral cancellous bone mass (Brunetti et al. 2018). However, the possible influence of *LIGHT* in muscle collagen synthesis and connective tissue development will require further study.

For the genes detected relating to 13 dpm collagen solubility, the *HSD11B1* (*hydroxysteroid 11-beta dehydrogenase 1*) gene encodes a microsomal enzyme that catalyzes the interconversion of the stress hormone cortisol to the inactive metabolite cortisone (Tomlinson et al. 2004). Research shows that inhibition of hydroxysteroid 11-beta dehydrogenase 1 may increase collagen content in aged skin and skin atrophy, indicating its role in connective tissue remodeling (Terao et al. 2014). By comparing the gene expression from normal skin and scar tissues, *HSD11B1* was found to be down-regulated in scar tissues, suggesting its possible

function in collagen synthesis and skin regeneration (Huang et al. 2015). The *LAMB3* (*laminin subunit beta 3*) gene encodes a protein called laminin belonging to a family of basement membrane proteins and laminin belongs to one of the active extracellular matrix molexules with about 40 active sites been identified (Kleinman et al. 2003). Together with tenascin-C, laminin can bind to epidermal growth factor receptors and enhance fibroblast migration (Tran et al. 2005). According to Waterman et al. (2007), through interactions with collagen VII, laminin can activate phosphoinositol-3-kinase and drives human epidermal carcinogenesis. The above discussion suggests the possible role of *LAMB3* gene in the development of connective tissue.

Based on the discussion above, some of the detected genes are related to blood vessels, bones, tendon and cartilage, and they did show some correlation with collagen, suggesting their possible roles in collagen synthesis and connective tissue development, although further studies are needed to test this conjecture. Some of the detected genes are not related to collagen directly based on comprehensive search of the gene functions from the literature, although they were involved in connective tissue development and function as shown in IPA results (Supplementary Table 5.5 to Supplementary Table 5.8), and this might be caused by imperfect gene annotations and imprecise GWAS region boundaries as discussed in Chapter 2. Generally, the significant SNPs identified in this study may play important roles in intramuscular total collagen content and/or collagen solubility, but further studies are needed focusing on the validation of those SNPs as well as validating the detected genes and searching for other candidate genes which may be related to collagen.

5.5. Conclusions

Based on our association study, 70 SNPs were detected for 3 dpm total collagen content using SNP windows that explained more than 1% of additive genetic variance, and 90 SNPs

were detected for 3 dpm collagen solubility, 100 SNPs and 60 SNPs were detected for 13 dpm total collagen content and collagen solubility, respectively. Functional annotation revealed that there were 5, 18, 19, and 3 genes that may be treated as candidate genes for 3 dpm total collagen, collagen solubility, 13 dpm total collagen, and collagen solubility, respectively. However, these results should be validated in a larger group of beef cattle before being considered for incorporation into future marker-assisted or genomic selection in beef cattle to improve beef tenderness through modification of IMCT.

Table 5.1. Least squares means (standard error) of the total collagen and collagen solubility (n = 137).

Trait	3 dpm	13 dpm	*p*-value
Total collagen content (mg/g)	5.72 (0.22)	4.95 (0.22)	0.014
Collagen solubility (%)	44.65 (1.54)	34.24 (1.50)	<0.0001

Note: Within a row for each trait, statistical significance threshold is $p < 0.05$ using a two-tailed t test.

Table 5.2. Summary of SNP windows (7) that explained > 1% of additive genetic variance for 3 dpm total collagen content.

Chromosome	SNP Window		Additive Genetic Variance (%)
	Start, BP	Stop, BP	
7	5866943	6206051	1.62
7	23074262	23568940	1.06
8	40159162	40610167	1.13
8	47092657	47437417	1.40
8	52474895	52939275	2.20
12	80707508	81184160	1.00
19	57748552	58091668	1.76

Table 5.3. Summary of SNP windows (9) that explained > 1% of additive genetic variance for 3 dpm collagen solubility.

Chromosome	SNP Window		Additive Genetic Variance (%)
	Start, BP	Stop, BP	
1	68507419	69045500	1.12
5	30061770	30476984	1.15
8	52303539	52808981	2.31
11	5060899	5349030	1.02
11	102885677	103289035	2.49
12	56289541	56695055	1.35
19	14673538	15060100	2.59
19	51326750	51680150	1.56
21	36945934	37293354	1.33

Table 5.4. Summary of SNP windows (10) that explained > 1% of additive genetic variance for 13 dpm total collagen content.

Chromosome	SNP Window		Additive Genetic Variance (%)
	Start, BP	Stop, BP	
2	79320427	79946595	1.01
2	129738866	130187033	1.35
3	8716142	9686101	2.38
7	18158398	18476120	1.59
7	65497498	65884166	1.02
8	46239174	46877924	1.16
11	41647520	42330073	1.07
19	34836416	35308991	2.13
20	6477168	6935534	1.31
27	22757505	23144459	1.51

Table 5.5. Summary of SNP windows (6) that explained > 1% of additive genetic variance for 13 dpm collagen solubility.

Chromosome	SNP Window		Additive Genetic Variance (%)
	Start, BP	Stop, BP	
4	81231390	81830858	1.14
6	12447020	12741496	1.34
7	37839380	38196134	1.23
11	12513910	13090203	1.36
14	79715760	80082923	1.09
16	73711818	74158269	1.60

Table 5.6. Possible candidate genes (5) for 3 dpm total collagen.

Categories	*p*-value	Genes
Connective Tissue Development and Function, Embryonic Development,Organ Development,Organismal Development,Skeletal and Muscular System Development and Function,Tissue Development	0.00231	*PCSK5*
Cell Cycle,Cellular Development,Connective Tissue Development and Function	0.00691	*SIN3B*
Cell Morphology,Connective Tissue Development and Function,Organismal Injury and Abnormalities	0.0183	*CDC42SE2*
Connective Tissue Development and Function,Tissue Morphology	0.0228	*PLVAP*
Cell-To-Cell Signaling and Interaction,Connective Tissue Development and Function	0.0318	*F2RL3*

Note: Note: *p*-value was generated using Fisher's exact test and $p < 0.05$ was set as significant.

Table 5.7. Possible candidate genes (18) for 3 dpm collagen solubility.

Categories	*p*-value	Genes
Cellular Movement,Connective Tissue Development and Function,Hepatic System Development and Function	0.000225	*CCL3,CCL4,CCL5*
Cell Morphology,Connective Tissue Development and Function	0.00235	*BAIAP2,RAC3*
Cellular Development,Cellular Growth and Proliferation,Connective Tissue Development and Function,Hematological System Development and Function,Hematopoiesis,Organismal Development,Tissue Development	0.0052	*SLFN14*
Connective Tissue Development and Function,Embryonic Development,Organ Development,Organismal Development,Skeletal and Muscular System Development and Function,Tissue Development	0.0052	*PCSK5*
Cell Morphology,Connective Tissue Development and Function	0.0052	*ARHGDIA*
Cellular Development,Cellular Growth and Proliferation,Connective Tissue Development and Function,Embryonic Development,Hematological System Development and Function,Hematopoiesis,Organismal Development,Tissue Development	0.0104	*GFI1B*
Cellular Development,Cellular Growth and Proliferation,Connective Tissue Development and Function,Hematological System Development and Function,Hematopoiesis,Organismal Development,Tissue Development	0.0239	*MAFG,SLFN14*
Cellular Development,Cellular Growth and Proliferation,Connective Tissue Development and Function	0.0308	*C1QL4*

Connective Tissue Development and Function,Organ Morphology,Organismal Development,Reproductive System Development and Function	0.0333	*GCGR,SIRT7*
Cellular Development,Cellular Growth and Proliferation,Connective Tissue Development and Function	0.0358	*ASPSCR1,C1QL4, MCRS1,RAC3,RALGDS,RPTOR,TSC1*

Table 5.8. Possible candidate genes (19) for 13 dpm total collagen.

Categories	*p*-value	Genes
Cellular Development,Cellular Growth and Proliferation,Connective Tissue Development and Function,Hematological System Development and Function,Lymphoid Tissue Structure and Development,Tissue Development	0.000086	*CD244,CD48*
Connective Tissue Development and Function	0.00437	*MSX2,RASD1,SREBF1*
Cell Cycle,Connective Tissue Development and Function	0.00565	*E2F2,ID3*
Cellular Development,Connective Tissue Development and Function	0.00779	*ENC1,KDM1A,MSX2,RASD1,SREBF1*
Cellular Development,Cellular Growth and Proliferation,Connective Tissue Development and Function,Tissue Development	0.00993	*MAP2K3,STAT1*
Connective Tissue Development and Function,Hematological System Development and Function,Humoral Immune Response,Lymphoid Tissue Structure and Development,Organismal Development,Tissue Morphology	0.0107	*TNFRSF13B*
Cellular Development,Connective Tissue Development and Function,Tissue Development	0.0121	*E2F2,MSX2*
Cellular Development,Connective Tissue Development and Function,Skeletal and Muscular System Development and Function,Tissue Development	0.0128	*C3,FCER1G,MAP2K3,STAT1,TNFSF14*

Connective Tissue Development and Function,Connective Tissue Disorders,Organismal Development,Organismal Injury and Abnormalities,Skeletal and Muscular Disorders,Skeletal and Muscular System Development and Function,Tissue Development	0.0138	*EPHB2,HEXB,LLGL1,MSX2,RAI1*

Table 5.9. Possible candidate genes (3) for 13 dpm collagen solubility.

Categories	*p*-value	Genes
Cellular Development,Connective Tissue Development and Function,Tissue Development	0.00578	*HSD11B1*
Cellular Development,Connective Tissue Development and Function,Tissue Development	0.0132	*DYSF,HSD11B1,LAMB3*

Figure 5.1. Manhattan plot of additive genetic variance explained by windows of 10 adjacent SNPs for 3 dpm total collagen content.

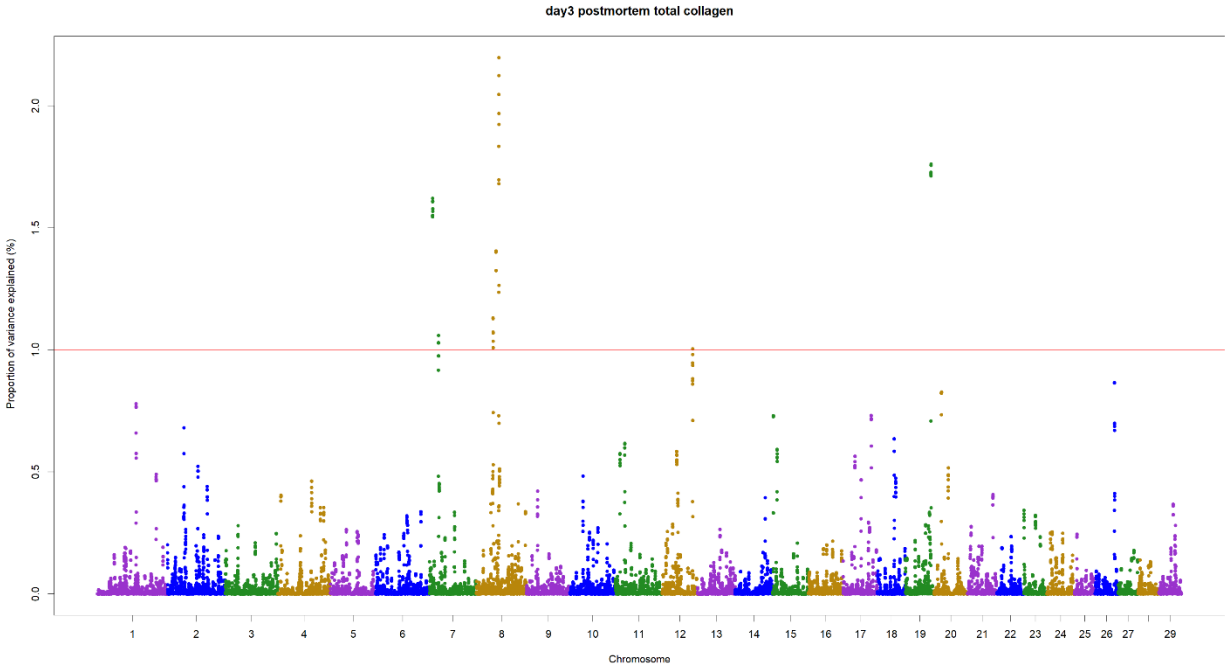


Figure 5.2. Manhattan plot of additive genetic variance explained by windows of 10 adjacent SNPs for 3 dpm collagen solubility.

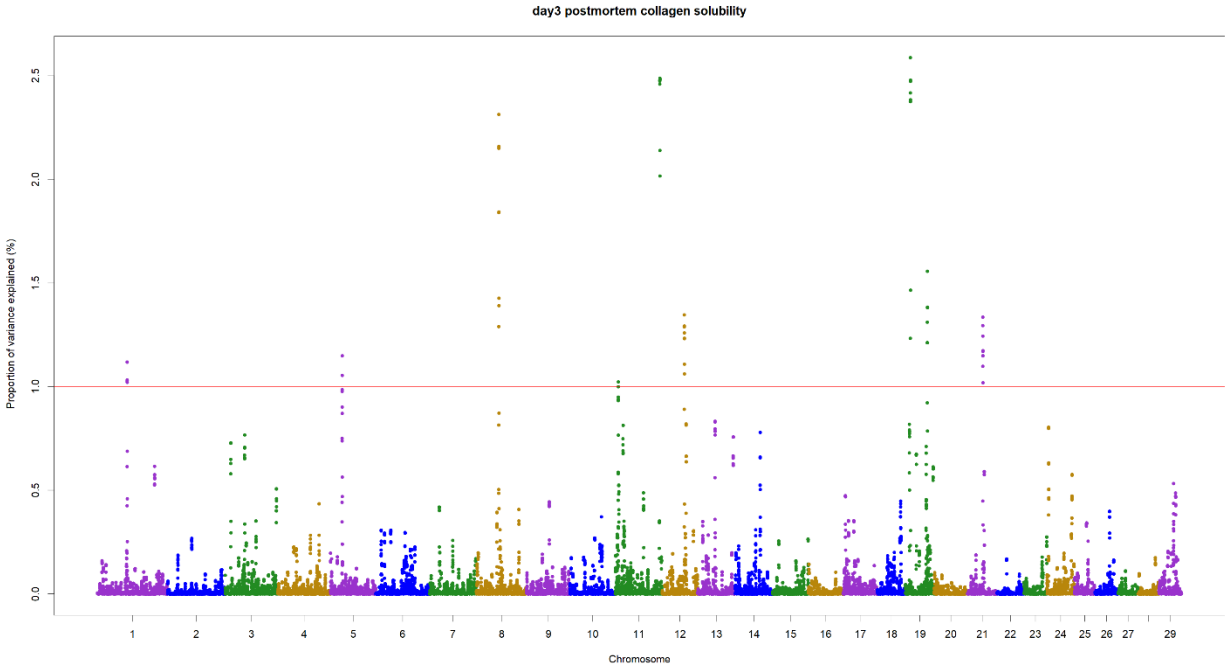


Figure 5.3. Manhattan plot of additive genetic variance explained by windows of 10 adjacent SNPs for 13 dpm total collagen content.

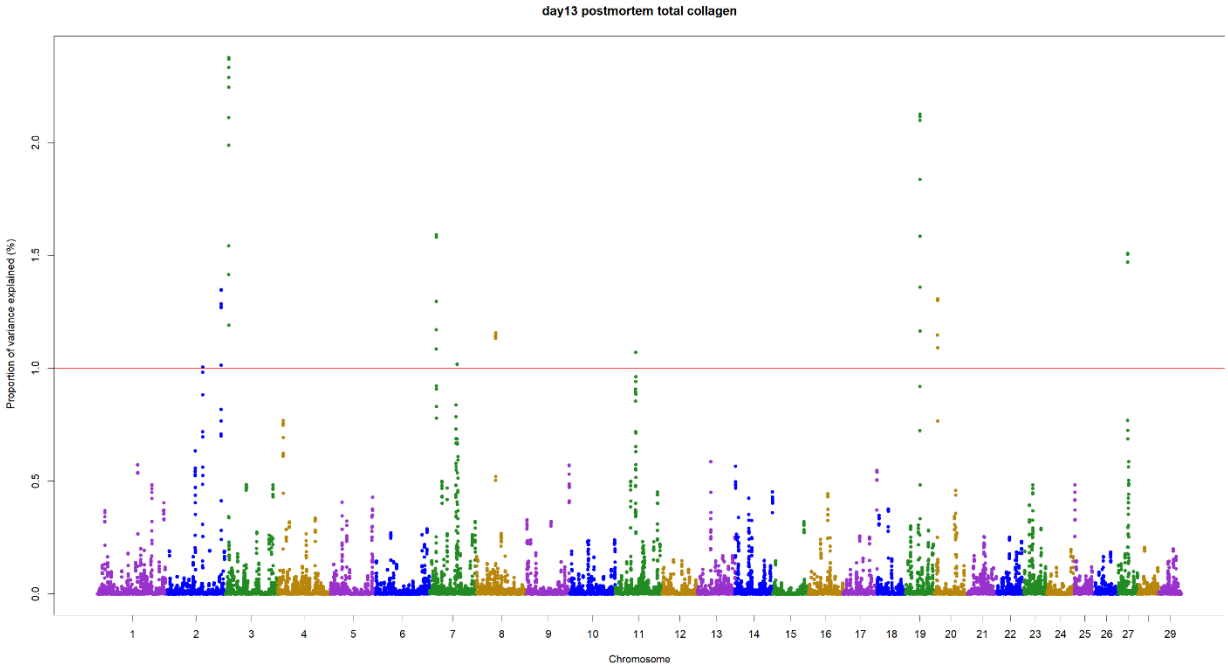


Figure 5.4. Manhattan plot of additive genetic variance explained by windows of 10 adjacent SNPs for 13 dpm collagen solubility.

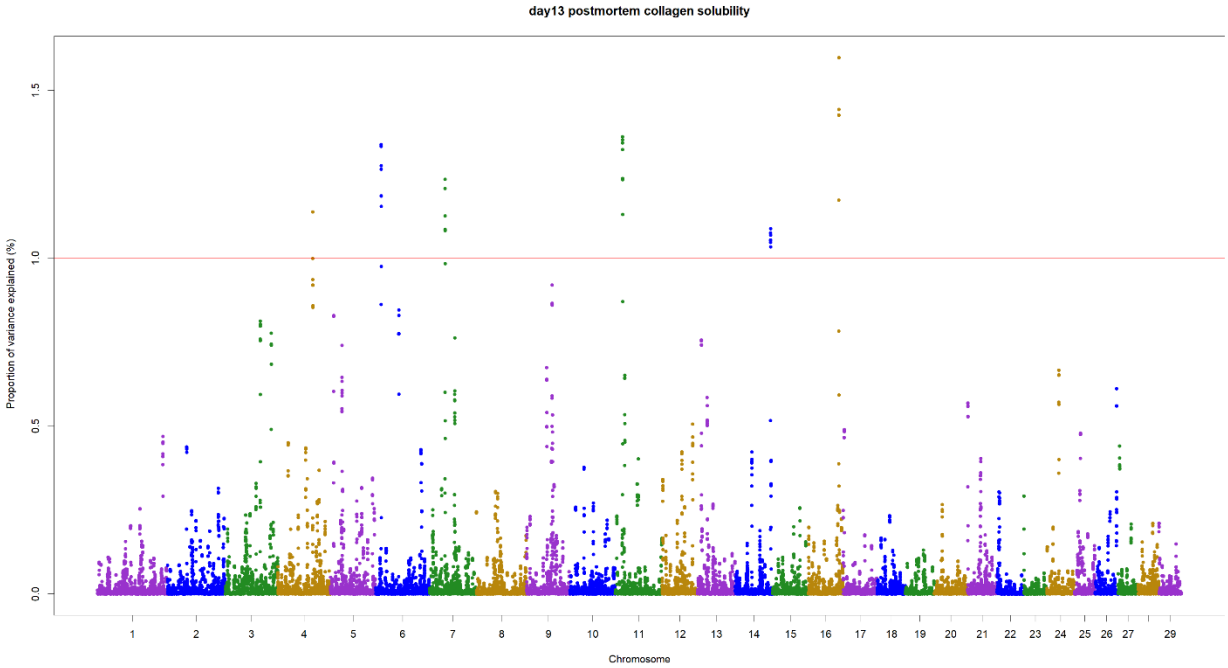
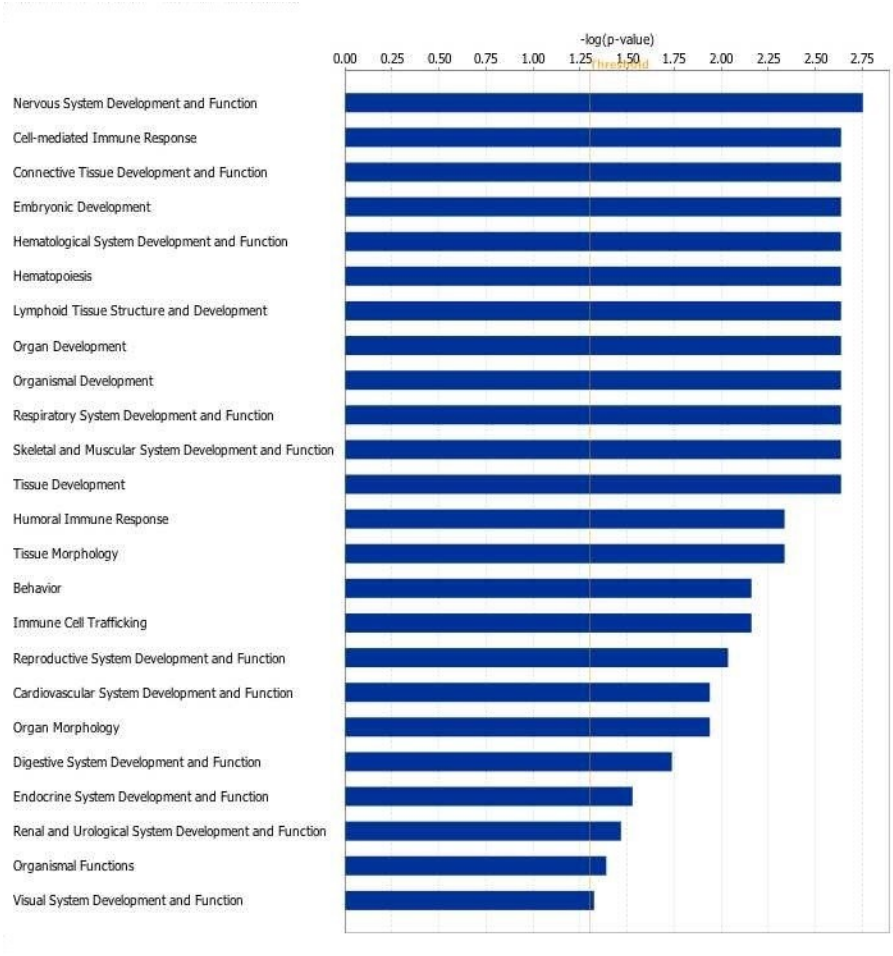


Figure 5.5. Physiological system development and function of the RefSeq genes harboring significant SNP windows for 3 dpm total collagen content.



Note: *p*-value was generated using Fisher's exact test and <0.05 was set as significant.

Figure 5.6. Physiological system development and function of the RefSeq genes harboring significant SNP windows for 3 dpm collagen solubility.

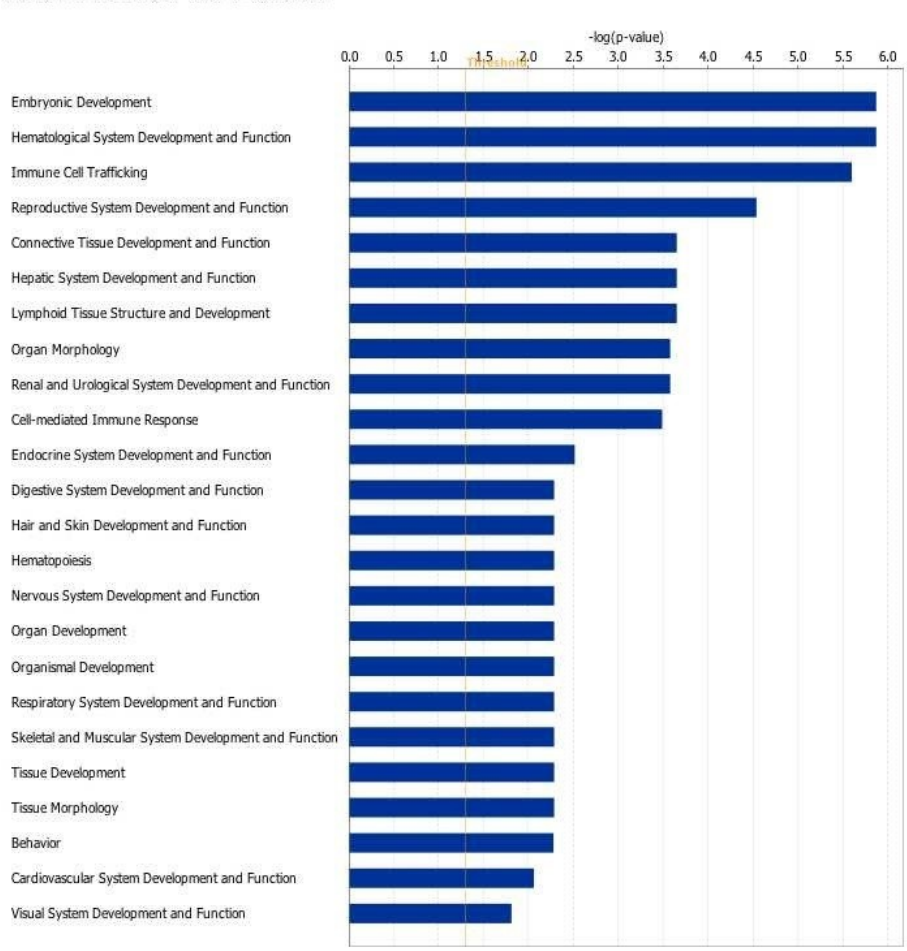


Figure 5.7. Physiological system development and function of the RefSeq genes harboring significant SNP windows for 13 dpm total collagen content.

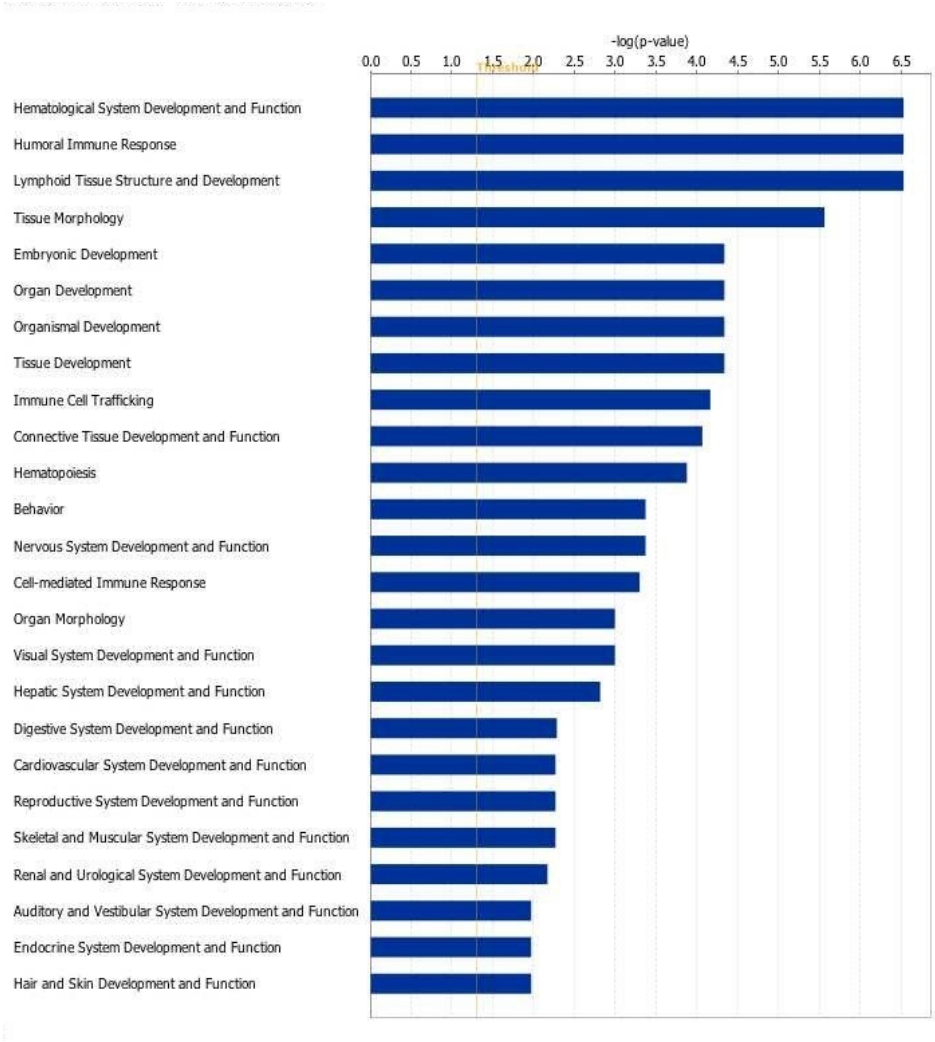
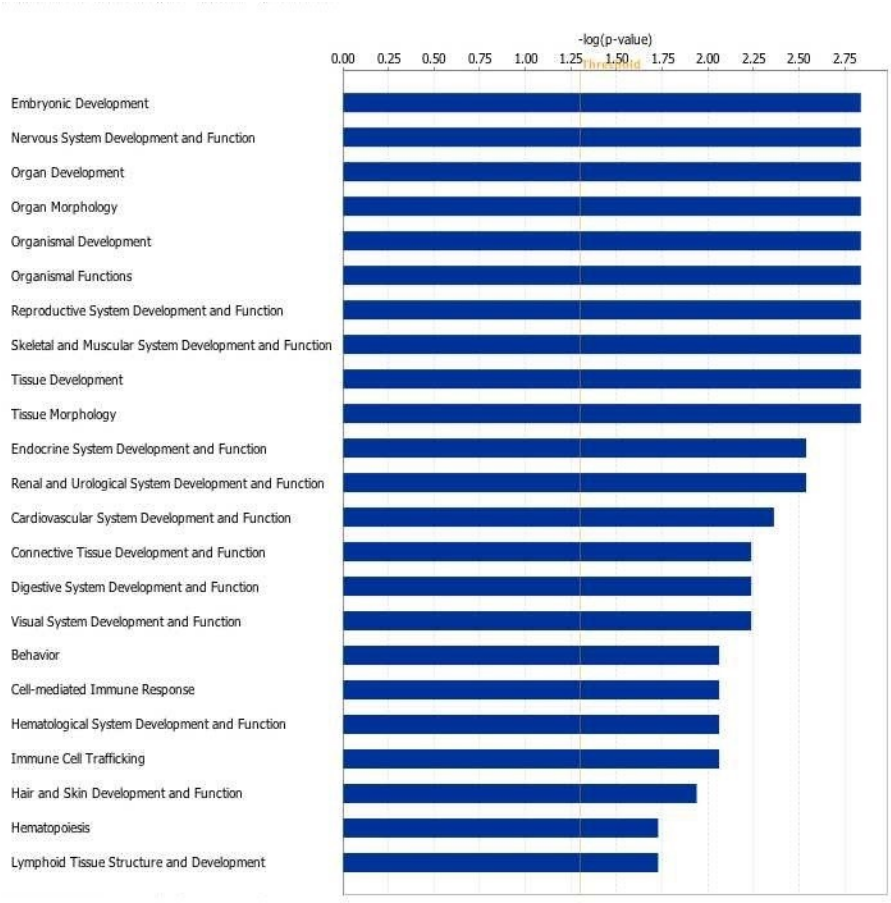


Figure 5.8. Physiological system development and function of the RefSeq genes harboring significant SNP windows for 13 dpm solubility.



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6. Summary and General Discussion

6.1. Overview

Living in a modern society, we as consumers are constantly pursuing a high quality of life by choosing a better diet. Meat, rich in proteins, vitamins and minerals, is essential for our health and is consumed very often by humans except for those who have certain religions or vegetarians. Besides the various nutrients meat provides us, consumers can experience eating satisfaction by consuming high quality meat. Meat quality attributes contributing to the satisfaction of meat include color, texture, juiciness and flavor (Listrat et al. 2016), and consumer satisfaction is the prerequisite of continuous meat consumption and consumer loyalty (Bearden and Teel 1983).

Among the meat quality attributes, intramuscular pH is also very important. In 1991, ultimate pH value was recognized as a primary postmortem factor influencing pork quality (Offer 1991) and according to Matarneh et al. (2017), the rate and extent of pH decline is an important influence on final meat quality attributes. The detailed explanation about the influence of pH on meat quality can be found in the *General Introduction* of this thesis. Abnormal meat pH usually indicates inferior meat, for example, as very high ultimate pH (pH > 5.8) is associated with the formation of dark cutting meat (Tarrant 1981), and dark cutting carcasses are down graded, causing non-negligible economic loss for beef producers and beef industry. Genetic selection of beef cattle tolerant of chronic stress may reduce the incidence of dark cutters as chronic stress experienced antemortem by cattle is the main cause of dark cutting beef, and SNP markers found in this study may be useful in marker-assisted selection or genomic selection of beef cattle to reduce dark cutting.

Besides meat pH, intramuscular fat content is also very important for meat quality. Using human senses, like sight, smell, touch, taste and hearing, to evaluate products is called sensory evaluation (Stone and Sidel 2004), which is a direct method accepted by researchers. To understand the influence of pH and intramuscular fat on pork chop sensory acceptability, frozen-thawed pork samples were used to estimate genetic parameters for sensory traits (Chapter 3). However, it is necessary to consider the influence of freezing on meat quality in genomic studies, which was addressed in Chapter 4 "*Efficacy of genetic parameter estimation of pork loin quality of crossbred commercial pigs using technological quality measurements of frozen and unfrozen product*". Due to the formation of ice crystals on muscle cells and organelle membranes during freezing of meat and the inability of water to be rebounded by the myofilaments during thawing of meat, many meat quality attributes (such as meat pH, color, drip loss, WBSF) may change if they go through freezing and then thawing process (Deatherage and Hamm 1960; Ngapo et al. 1999; Lagerstedt et al. 2008; Vieira et al. 2009; Leygonie et al. 2012).

Another widely studied meat quality attribute is meat tenderness, determined by the combination of myofibrillar proteins, intramuscular fat and connective tissue (Van Laack et al. 2001). Connective tissue, which consists mainly of the proteins collagen and elastin, is responsible for cooked meat background toughness and it is difficult to improve meat tenderness by reducing the toughness caused by connective tissue through traditional methods like aging. Genetic selection of animals with less collagen or high soluble collagen may be useful to increase meat tenderness.

6.2. Genetics of dark cutting in beef cattle

The first study (Chapter 2) was executed to investigate if there are SNPs that are associated with dark cutting beef and if the genes nearby these SNPs are biologically relevant to

the formation of dark cutting beef. In this study, two groups of beef cattle were used and dark-cutting beef was analyzed as a binary trait (cases versus controls) using logistic regression under an additive model. The GWAS was done on Group I, Group II and the Combined Group separately and detected 449 significant SNPs in Group 1, 301 in Group II, and 209 in Combined Groups using a relatively relaxed statistical threshold of $p < 0.01$. Those SNPs were used to check the nearby RefSeq genes through Ensembl BioMart and the results showed that the genes may have influence on the formation of dark cutting beef. Based on the nominal p values, no strong evidence was found for a large influence of any one SNP in the incidence of dark cutting beef, suggesting that the trait may be polygenic. However, based on the functional analysis, the most significant SNPs did show suggestive association with dark cutting beef as the nearest genes had biological relevance to carbohydrate metabolism, suggesting the possibility in the future for marker-assisted or genomic selection in beef cattle to reduce dark cutting.

6.3. Influence of meat pH and intramuscular fat in pork sensory attributes

The second study aimed to examine the influence of intramuscular pH and fat content on pork chop sensory acceptability. The heritabilities of pork sensory attributes as well as the phenotypic and genetic correlations of important sensory traits with pH and intramuscular fat content were estimated in that study. Both univariate and bivariate animal models were used for the analysis in ASReml. Low heritability was found for all sensory attributes, suggesting that environmental factors (including the freezing and thawing process) obscured genetic effects. Low to moderate genetic correlations were detected between pH and sensory traits, and moderate to high genetic correlations with sensory measurements were estimated, which indicated the importance of intramuscular crude fat to the sensory attributes compared with meat pH in product that had been frozen and then thawed, suggesting that selecting pigs with increased intramuscular fat may increase consumer acceptance compared to selection for meat ultimate pH

in this sort of product. These results cannot be applied to fresh pork, as intramuscular pH may be of increased importance in unfrozen pork or meat products. This paradigm should be considered by researchers, but the effects of sample handling on phenotype expression has not been widely recognized in genetic studies, hence it exploration in this thesis in Chapter 4.

6.4. Influence of freezing and then thawing process on meat quality

The study detailed in Chapter 4 aimed to estimate the heritability of important meat quality traits measured using fresh and frozen-thawed pork, and the phenotypic, genetic and environmental correlations of meat quality measurements of fresh and frozen-thawed product, as well as to evaluate the effect of crude fat content on meat quality measurements in fresh and frozen-thawed pork. A bivariate animal model in ASReml was used for genetic parameter estimation. The results showed that meat quality traits measured before and after freezing and thawing were significantly (P<0.0001) different from each other and intramuscular crude fat content exerted a large effect on the magnitude of change in L* and b*. Meat quality measurements of fresh pork were moderately to highly heritable except for b* and pH, with heritability estimates for L*, pH and drip loss higher when measured on fresh rather than frozen-thawed samples. Considering heritability and genetic correlation results, conclusions have been made that whilst either fresh or frozen-thawed pork samples can be used for L*, a* and b* measurements, pH and possibly drip loss should be measured in fresh pork samples rather than in those that have been frozen-thawed for genetic selection.

6.5. Genetics of collagen in beef cattle

The study detailed in Chapter 5 was executed to identify variations (e.g.: single nucleotide polymorphisms (SNPs)) in genes along the genome associated with total collagen and collagen solubility, and to explore the biological relevance of the genes to beef toughness caused by IMCT. The estimation of variance components and GWAS were performed by using single-

step Genomic Best Linear Unbiased Prediction (ssGBLUP), and the final results were reported as the proportion of genetic variance explained by a consecutive sliding window of 10 adjacent SNPs. In total, 7 SNP windows that explained more than 1% of additive genetic variance were detected for 3 dpm total collagen content and 9 SNP windows for 3 dpm collagen solubility. For 13 dpm, 10 and 6 SNP windows were detected for total collagen and collagen solubility, respectively. Functional annotation revealed that there were 5, 18, 19, and 3 genes may be treated as candidate genes for 3 dpm total collagen, collagen solubility, 13 dpm total collagen, and collagen solubility, respectively. A very small population of cattle was used in these studies, limiting the confidence in these results. Analysis of collagen characteristics is laborious, and therefore populations involving these measurements are often small. Large databases involving collagen characteristics are usually generated by amalgamating many small studies together to achieve the data numbers required for some level of confidence to be achieved. As a result, the associations observed in this thesis should be validated using increased numbers of cattle before they can be considered for marker-assisted or genomic selection in beef cattle to improve beef tenderness through IMCT in the future.

6.6. Limitations and future implications

The findings that there are SNPs associated with dark cutting beef along the genome, and that their nearby genes are involved in glycogen and glucose metabolism, suggests the possibility of marker-assisted or genomic selection in the future to reduce dark cutting in beef cattle. However, these results need to be validated in a larger group of cattle than was available to this thesis before they can be considered for confirmation using other methods like RNA-Seq or qPCR. Based on the functional analysis, there is a possibility that some genes may be involved in the formation of dark cutting beef so it can be hypothesized that these genes are differentially expressed between dark cutters and normal carcasses, which can be confirmed using a qPCR

strategy. The major limitation of studying the influence of genetic on dark cutting beef is the difficulty of obtaining sufficient "case" or dark cutting samples, as the occurrence of dark cutters is very unpredictable. In this thesis, dark cutting carcasses were identified and harvested post mortem, and stressing cattle to the point of dark cutting is of questionable ethics. Multiple loading and unloading events have been linked to high percentages of dark cutting, and ante mortem or early post mortem sampling of muscle in cattle in a controlled experiment that have experienced these events could provide additional understanding of the relationship between the occurrence of dark cutting and the genome. RNA-Seq couold then be used to characterize the muscle from this experiment. Routine collection of muscle in slaughterhouses would quickly provide a large database to relate Canada B4 grade to the genome, but little phenotypic information would be available beyond grade information and slaughter and carcass weights, which are already known to have some relationship with the incidence of dark cutting. Future research can also use RNA-Seq technology to screen up and down regulated genes in order to better understand dark cutting related genes.

Based on the low heritability found in Chapter 3 for all sensory attributes, it can be suggested that managing the environmental factors that affect pork quality may be more efficient than genetically selecting pigs for pork chop acceptance, as management is usually used as the primary tool for improving performance in less heritable traits (Bourdon 2000). However, because of the importance of pork sensory attributes, they may deserve to be selected genetically regardless of their relatively low heritability. One way to genetically improve low heritable traits like sensory traits is through genomic selection, as breeding values can be predicted with high accuracy using dense SNP markers (Goddard and Hayes 2007). With high accuracy of breeding values, genetic gain can be increased.

Freezing meat for transportation and storage is very common nowadays, and the tremendous meat quality changes from fresh to frozen-thawed need to be considered. In Study 3 (Chapter 4), only a few important meat quality characteristics (pH, color, and drip loss) were evaluated. Based on previous research (Deatherage and Hamm 1960; Ngapo et al. 1999; Lagerstedt et al. 2008; Vieira et al. 2009; Leygonie et al. 2012), other meat quality attributes will also be changed during the process of freezing and thawing, which begs the hypothesis that the genetic correlation of those meat quality traits measured on fresh pork is higher than that of meat quality measured on frozen-thawed pork. The results presented in this thesis are the first to show the importance of using fresh meat rather than frozen product to obtain relevant phenotypic results for selection of pigs for fresh meat quality. Future research may focus on the influence of freezing and thawing on other meat quality traits and should ensure that these meat quality attributes are measured using the same methods for both fresh and frozen-thawed product.

Results from the collagen GWAS showed that there are SNPs that are associated with total collagen content and collagen solubility. The nearby RefSeq genes are also worthy of validation as they are related to connective tissue development and function. A good way to validate the genes is to measure the relative expression level of those genes through qPCR comparing samples from high total collagen and collagen solubility with low total collagen and collagen solubility. To date, there is little genetic research about IMCT total collagen and collagen solubility in terms of meat toughness, and one reason might be the difficulty in obtaining phenotypic records. However, the GWAS results also need to be validated in a larger population than that described in this thesis as the sample size was relatively small in this study and the methodology used to quantify collagen in skeletal muscle was complicated, which may

have obscured any genetic effects. Despite these challenges, genes for further research were identified, and these genes will provide new hypotheses for future research.

6.7. Overall conclusion

Based on the four independent yet interconnected studies, a series of conclusions were made that suggested the possiblility of future genetic selection of farm animals to produce superior meat through animal breeding, focusing on meat colour, sensory traits, intramuscular fat, and tenderness. These studies collectively indicated that there is no single gene that controls meat colour or toughness or connective tissue. They do indicate that multiple genes contribute to the various aspects of fresh meat and its cooked quality, and this is useful in that it provides us with a way forward in the discovery of how meat quality develops and can be controlled. Any genes or SNPs associated with these characteristics, however, need further validation with large numbers of animals before they can be incorporated into breeding programs, and the meat quality traits examined in this thesis are only a few in term of the overall consumer perception of meat. Hence, additional comprehensive research should be performed to deeply understand how genetic selection can be used to meet the needs of meat consumers and provide better products for meat consumers.

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Appendix A

Consumer Evaluation of Pork – Demographics Questionnaire

Information about Yourself Participant # _____

1. Please indicate your gender:

❒ Male ❒ Female

2. Please indicate the age group that you belong to:

❒ 18-29 years ❒ 50-59 years
❒ 30-39 years ❒ 60-69 years
❒ 40-49 years ❒ 70 years plus

3. Please indicate how many people live in your household:

❒ 1 person ❒ 4 persons
❒ 2 persons ❒ 5 or more persons
❒ 3 persons

4. Where do you normally purchase **pork products**? (Please circle the number that best represents your purchasing habits)

	Always	Most Often	Sometimes	Rarely	Never
Supermarkets (*e.g.* Save-On, Safeway)	1	2	3	4	5
Meat Shops (*e.g.* M&M Meat Shops)	1	2	3	4	5
Farmers' Markets	1	2	3	4	5
Wholesalers (*i.e.* Costco)	1	2	3	4	5
Other: (please specify)	1	2	3	4	5

5. Which of the following foods would you consider to be your favourite?

❒ Beef/Veal ❒ Lamb/Sheep/Mutton
❒ Bison ❒ Fish/Seafood
❒ Chicken ❒ Meatless meal
❒ Turkey ❒ None of the above
❒ Pork

6. On average, how often do you eat pork?

❒ More than 3 times a week
❒ 2 – 3 times a week
❒ Once a week
❒ Every 2 – 3 weeks
❒ Once a month
❒ Never

Appendix B

Sensory Evaluation Form

Study Number: NSERC Pork CRD

Date: _____ Time: _____

Participant # _____ Location: _____

Sample # _____

1. Overall, what is your opinion of the **texture** of the pork sample?

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

2. The **texture** of this pork sample is…..

☐	☐	☐	☐	☐
Much too tough	Slightly too tough	Just about right	Slightly too tender	Much too tender

3. The **juiciness** of this pork sample is…..

☐	☐	☐	☐	☐
Much too dry	Slightly too dry	Just about right	Slightly too juicy	Much too juicy

4. Overall, what is your opinion of the **flavour** of the pork sample?

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

5. What is your **overall opinion** of this pork sample?

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

6. How similar is this sample to your ideal?

1	2	3	4	5	6	7	8	9	10
Completely different			Very different			Somewhat different			Matches my ideal

7. What did you like or dislike about this pork sample?

Appendix C

Supplementary Table 2.1. Fifty (50) associated SNPs with the lowest *p* values in imputed data.

Ranking	SNP ID	Chromosome	Position	p-value	HB_FDR
1	BovineHD0900017222	9	62655387	7.88E-06	0.407317
2	ARS-BFGL-NGS-101411	2	72163562	1.21E-05	0.407317
3	ARS-USMARC-Parent-EF034081-rs29009668	13	25606469	0.000141	0.954578
4	BTB-00388242	9	34426533	0.000182	0.954578
5	BTB-00436535	10	84388256	0.000207	0.954578
6	ARS-BFGL-NGS-39532	8	10623685	0.000235	0.954578
7	BovineHD0900009429	9	34392779	0.000273	0.954578
8	BovineHD2700006373	27	22593244	0.000285	0.954578
9	BovineHD1900015453	19	54989339	0.000299	0.954578
10	UA-IFASA-7071	19	23628220	0.000324	0.954578
11	BovineHD0800023965	8	80167679	0.000359	0.954578
12	UA-IFASA-5538	6	58871346	0.000365	0.954578
13	BovineHD2100001500	21	7075853	0.000369	0.954578
14	BovineHD2100001525	21	7109497	0.000369	0.954578
15	BovineHD2100001542	21	7165261	0.000369	0.954578
16	Hapmap46736-BTA-94583	16	10740538	0.000416	0.954578
17	BovineHD2100001480	21	7049231	0.000454	0.954578
18	ARS-BFGL-NGS-39984	17	72414626	0.000482	0.954578
19	BovineHD0200039339	2	1.35E+08	0.000517	0.954578
20	BovineHD4100010595	14	4018136	0.000522	0.954578
21	ARS-BFGL-NGS-19602	14	21286894	0.000584	0.954578
22	BovineHD0100001164	1	3515994	0.00059	0.954578
23	BTB-01762552	8	37192348	0.000592	0.954578
24	BovineHD1200005501	12	18168991	0.000596	0.954578
25	BovineHD0600033631	6	1.18E+08	0.000616	0.954578
26	BovineHD0200004852	2	17043859	0.000654	0.954578
27	BTA-121196-no-rs	19	59441263	0.000665	0.954578
28	BovineHD1100015033	11	51119421	0.00069	0.954578
29	BTB-01108785	4	21118823	0.000764	0.954578
30	Hapmap39358-BTA-82003	8	80126915	0.000764	0.954578
31	BTB-00244579	6	13502824	0.000771	0.954578
32	BovineHD1300007383	13	25499686	0.000789	0.954578
33	BTB-00934994	29	23935266	0.000795	0.954578
34	BovineHD0200020657	2	71995785	0.000801	0.954578
35	Hapmap25127-BTA-83222	9	34658951	0.000817	0.954578
36	Hapmap57169-rs29020666	8	9009550	0.000818	0.954578
37	Hapmap55314-rs29026474	24	52825393	0.000822	0.954578
38	ARS-BFGL-NGS-21302	13	55973589	0.000824	0.954578
39	ARS-BFGL-NGS-42736	3	33367402	0.000838	0.954578

40	BovineHD0100034683	1	1.23E+08	0.000872	0.954578
41	BovineHD0900000335	9	1709172	0.000893	0.954578
42	ARS-BFGL-NGS-5833	9	54567712	0.000905	0.954578
43	BovineHD1600007188	16	25861658	0.000925	0.954578
44	ARS-BFGL-NGS-78497	24	21330516	0.000926	0.954578
45	Hapmap53690-rs29026074	24	53195202	0.000929	0.954578
46	BovineHD0900002737	9	11019894	0.000974	0.954578
47	BovineHD0900002738	9	11022138	0.000974	0.954578
48	BovineHD0900002740	9	11024991	0.000974	0.954578
49	BovineHD2300014425	23	49462505	0.000988	0.954578
50	BTA-37329-no-rs	15	66868403	0.001017	0.954578

Appendix D

Supplementary Table 3.1. Descriptive statistics of all the sensory measurements.

Group	pH	fat	lsmean	SE	df	lower.CL	upper.CL[a]	pH effect	fat effect	interaction
Overall Texture								Pr > (F)	Pr > (F)	Pr > (F)
G1	low	low	5.64	0.10	780	5.44	5.84			
G2	low	high	5.72	0.06	780	5.60	5.85	0.48	0.29	0.97
G3	high	low	5.59	0.09	780	5.41	5.76			
G4	high	high	5.68	0.06	780	5.55	5.80			
Texture								Pr > (F)	Pr > (F)	Pr > (F)
G1	low	low	2.41	0.03	780	2.36	2.47			
G2	low	high	2.47	0.03	780	2.41	2.53	0.04	0.17	0.71
G3	high	low	2.35	0.04	780	2.27	2.43			
G4	high	high	2.44	0.05	780	2.34	2.53			
Juiciness								Pr > (F)	Pr > (F)	Pr > (F)
G1	low	low	2.45	0.03	779	2.40	2.50			
G2	low	high	2.43	0.03	779	2.38	2.48	0.05	0.22	0.35
G3	high	low	2.42	0.04	779	2.35	2.49			
G4	high	high	2.44	0.04	779	2.36	2.52			
Flavour								Pr > (F)	Pr > (F)	Pr > (F)
G1	low	low	5.86	0.05	780	5.75	5.96			
G2	low	high	5.87	0.05	780	5.77	5.98	0.97	0.09	0.5
G3	high	low	5.78	0.07	780	5.64	5.93			
G4	high	high	5.71	0.09	780	5.54	5.88			
Overall Opinion								Pr > (F)	Pr > (F)	Pr > (F)
G1	low	low	5.65	0.06	780	5.53	5.76			
G2	low	high	5.68	0.06	780	5.56	5.79	0.73	0.08	0.76
G3	high	low	5.53	0.08	780	5.37	5.70			
G4	high	high	5.52	0.10	780	5.33	5.71			
Similar to Ideal								Pr > (F)	Pr > (F)	Pr > (F)
G1	low	low	6.41	0.07	780	6.27	6.54			
G2	low	high	6.40	0.07	780	6.26	6.53	0.7	0.18	0.58
G3	high	low	6.33	0.10	780	6.14	6.52			
G4	high	high	6.23	0.11	780	6.01	6.44			

[a] Confidence level: 95% was used.

Appendix E

Supplementary Table 5.1. SNPs (70) within the seven significant windows for 3 dpm total collagen.

SNPID	Chromosome	Position (bp)
ARS-BFGL-NGS-39011	7	5866943
BTB-01746209	7	5903128
ARS-BFGL-NGS-32423	7	5955666
Hapmap49762-BTA-80425	7	6010779
ARS-BFGL-NGS-55814	7	6036526
ARS-BFGL-NGS-87888	7	6068869
ARS-BFGL-NGS-67299	7	6097996
ARS-BFGL-NGS-101854	7	6134663
ARS-BFGL-NGS-26591	7	6166179
ARS-BFGL-NGS-117791	7	6206051
Hapmap51514-BTA-88337	7	23074262
ARS-BFGL-NGS-112444	7	23275178
ARS-BFGL-NGS-118596	7	23315210
ARS-BFGL-NGS-112246	7	23336356
BTA-78609-no-rs	7	23377738
Hapmap57528-rs29013765	7	23403749
BTB-00301021	7	23426104
Hapmap57260-rs29023390	7	23498152
ARS-BFGL-NGS-67859	7	23519206
ARS-BFGL-NGS-76969	7	23568940
ARS-BFGL-NGS-6430	8	40159162
ARS-BFGL-NGS-105204	8	40228332
Hapmap52197-rs29019858	8	40282188
ARS-BFGL-NGS-63764	8	40306760
Hapmap27456-BTA-158380	8	40389762
ARS-BFGL-NGS-8821	8	40422559
Hapmap36155-SCAFFOLD226597_1989	8	40470452
Hapmap59547-rs29026130	8	40538141
Hapmap33220-BTA-149236	8	40585048
Hapmap33243-BTA-158375	8	40610167
Hapmap25377-BTA-84400	8	47092657
BTA-111112-no-rs	8	47138456
ARS-BFGL-NGS-118200	8	47179793
BTB-01127160	8	47224522
ARS-BFGL-NGS-116077	8	47266632
Hapmap42727-BTA-92113	8	47313169
BTB-01127329	8	47336496

BTB-01415880	8	47366278
BTA-90958-no-rs	8	47412695
ARS-BFGL-NGS-97065	8	47437417
BTB-00348223	8	52474895
BTB-00795717	8	52557297
BTB-00348139	8	52645428
Hapmap40911-BTA-121841	8	52693230
BTA-81190-no-rs	8	52724898
Hapmap47747-BTA-81189	8	52780433
Hapmap48089-BTA-81187	8	52808981
ARS-BFGL-NGS-106217	8	52857436
BTB-00348080	8	52912873
ARS-BFGL-NGS-76537	8	52939275
ARS-BFGL-NGS-32310	12	80707508
ARS-BFGL-NGS-27810	12	80804251
ARS-BFGL-NGS-75100	12	80851441
UA-IFASA-2080	12	80892109
Hapmap48943-BTA-91147	12	80915244
BTA-91151-no-rs	12	80978461
ARS-BFGL-NGS-22985	12	81048745
ARS-BFGL-NGS-5118	12	81084869
ARS-BFGL-NGS-8073	12	81119950
ARS-BFGL-NGS-13252	12	81184160
UA-IFASA-6080	19	57748552
UA-IFASA-6546	19	57770336
ARS-BFGL-NGS-115987	19	57797006
ARS-BFGL-NGS-33946	19	57832194
UA-IFASA-7954	19	57946240
ARS-BFGL-NGS-28163	19	57980697
ARS-BFGL-NGS-29724	19	58024390
UA-IFASA-7571	19	58038747
ARS-BFGL-NGS-21414	19	58059730
ARS-BFGL-NGS-33473	19	58091668

Appendix F

Supplementary Table 5.2. SNPs (90) within the nine significant windows for 3 dpm collagen solubility.

SNPID	Chromosome	Position (bp)
ARS-BFGL-NGS-9429	1	68507419
Hapmap50666-BTA-34589	1	68533156
ARS-BFGL-NGS-72247	1	68579776
Hapmap39647-BTA-34584	1	68604562
ARS-BFGL-NGS-31379	1	68891058
ARS-BFGL-NGS-19470	1	68937163
Hapmap59979-rs29025402	1	68966218
ARS-BFGL-NGS-35021	1	68997018
ARS-BFGL-NGS-26771	1	69022384
ARS-BFGL-NGS-100745	1	69045500
Hapmap34759-BES10_Contig780_1565	5	30061770
BTA-06718-no-rs	5	30085168
Hapmap47089-BTA-73292	5	30114907
ARS-BFGL-NGS-112542	5	30159843
ARS-BFGL-NGS-119788	5	30185840
Hapmap39286-BTA-73191	5	30275164
ARS-USMARC-635	5	30353509
ARS-USMARC-633	5	30374517
Hapmap52787-rs29024515	5	30417623
ARS-BFGL-NGS-15492	5	30476984
BTB-00348409	8	52303539
ARS-BFGL-NGS-65269	8	52348205
ARS-BFGL-NGS-73651	8	52432552
BTB-00348223	8	52474895
BTB-00795717	8	52557297
BTB-00348139	8	52645428
Hapmap40911-BTA-121841	8	52693230
BTA-81190-no-rs	8	52724898
Hapmap47747-BTA-81189	8	52780433
Hapmap48089-BTA-81187	8	52808981
Hapmap53281-rs29026129	11	5060899
ARS-BFGL-NGS-77332	11	5095286
BTB-01118640	11	5129135
ARS-BFGL-NGS-114076	11	5152170
Hapmap53318-rs29018775	11	5209460
ARS-BFGL-NGS-68850	11	5252617
BTB-01118763	11	5281215
BTB-01118794	11	5290404

BTA-101050-no-rs	11	5313647
ARS-BFGL-NGS-43582	11	5349030
ARS-BFGL-NGS-28626	11	102885677
BTA-119672-no-rs	11	102911946
ARS-BFGL-NGS-37871	11	102932140
ARS-BFGL-NGS-119318	11	102974570
ARS-USMARC-Parent-AY851163-rs17871661	11	103047474
ARS-BFGL-NGS-119907	11	103055283
ARS-BFGL-NGS-115328	11	103110855
ARS-BFGL-NGS-71542	11	103164589
ARS-BFGL-NGS-33009	11	103264921
ARS-BFGL-NGS-31097	11	103289035
BTB-01021521	12	56289541
BTB-01021469	12	56326750
Hapmap51019-BTA-65454	12	56367819
ARS-BFGL-NGS-104509	12	56380581
ARS-BFGL-NGS-96681	12	56418392
BTB-01834845	12	56553579
BTB-00499460	12	56586007
Hapmap43219-BTA-26954	12	56623014
BTA-26956-no-rs	12	56653121
BTB-00499591	12	56695055
ARS-BFGL-NGS-113158	19	14673538
ARS-BFGL-NGS-21921	19	14715567
ARS-BFGL-NGS-103651	19	14742543
ARS-BFGL-NGS-30310	19	14763072
Hapmap59291-rs29022102	19	14815755
ARS-BFGL-BAC-33086	19	14853169
ARS-BFGL-NGS-57566	19	14923593
Hapmap40357-BTA-46514	19	15000767
Hapmap41549-BTA-46518	19	15038933
UA-IFASA-6550	19	15060100
ARS-BFGL-NGS-39328	19	51326750
ARS-BFGL-NGS-20701	19	51371815
ARS-BFGL-NGS-39983	19	51395684
ARS-BFGL-NGS-73980	19	51419352
Hapmap42556-BTA-45815	19	51445450
ARS-BFGL-NGS-15454	19	51478494
ARS-BFGL-NGS-35888	19	51507347
ARS-BFGL-NGS-104734	19	51538272
ARS-BFGL-NGS-90673	19	51581082
ARS-BFGL-NGS-117290	19	51680150
BTB-01525993	21	36945934
BTB-01303932	21	36972268

BTB-01303945	21	36993382
BTB-01303893	21	37026202
BTB-01303877	21	37065628
BTB-01303847	21	37088797
BTB-01303836	21	37111472
BTB-01303828	21	37143433
BTB-01303818	21	37233645
BTB-01303761	21	37293354

Appendix G

Supplementary Table 5.3. SNPs (100) within the ten significant windows for 13 dpm total collagen.

SNPID	Chromosome	Position (bp)
Hapmap33049-BTA-153946	2	79320427
ARS-BFGL-NGS-33744	2	79388083
ARS-BFGL-NGS-65317	2	79414331
Hapmap39881-BTA-105105	2	79467938
ARS-BFGL-NGS-117618	2	79491364
ARS-BFGL-NGS-112785	2	79527086
Hapmap43710-BTA-86183	2	79846105
ARS-BFGL-NGS-94630	2	79893482
Hapmap35064-BES1_Contig468_700	2	79923716
Hapmap51332-BTA-86182	2	79946595
ARS-BFGL-NGS-116941	2	129738866
ARS-BFGL-NGS-118140	2	129768904
ARS-BFGL-NGS-116403	2	129845311
ARS-BFGL-NGS-36151	2	129927678
ARS-BFGL-NGS-2341	2	129947923
ARS-BFGL-NGS-102007	2	130031069
Hapmap39569-BTA-49765	2	130053163
ARS-BFGL-NGS-90021	2	130076674
BTA-49769-no-rs	2	130141723
ARS-BFGL-NGS-33709	2	130187033
ARS-BFGL-NGS-30351	3	8716142
ARS-BFGL-NGS-33910	3	8945826
BTA-93783-no-rs	3	8964885
Hapmap39362-BTA-95002	3	9077425
ARS-BFGL-NGS-113990	3	9461404
ARS-BFGL-NGS-108869	3	9528564
INRA-304	3	9579325
ARS-BFGL-NGS-18442	3	9610758
ARS-BFGL-NGS-105595	3	9658384
ARS-BFGL-NGS-1014	3	9686101
ARS-BFGL-NGS-105928	7	18158398
Hapmap53193-rs29010998	7	18199979
Hapmap60436-ss46526689	7	18283528
Hapmap31439-BTA-144800	7	18303700
ARS-BFGL-NGS-34826	7	18356430
Hapmap35741-SCAFFOLD181588_9953	7	18379589
ARS-BFGL-NGS-111581	7	18419552
ARS-USMARC-Parent-DQ786758-	7	18454636

rs29024430		
Hapmap36218-SCAFFOLD41765_2717	7	18454636
Hapmap49757-BTA-78451	7	18476120
BTB-01219349	7	65497498
ARS-BFGL-NGS-33898	7	65520210
Hapmap36214-SCAFFOLD145184_7453	7	65577310
ARS-BFGL-NGS-89122	7	65636193
BTB-01219206	7	65662751
ARS-BFGL-NGS-66769	7	65689219
ARS-BFGL-NGS-14780	7	65761014
BTB-01219012	7	65799159
ARS-BFGL-NGS-113819	7	65829695
ARS-BFGL-NGS-109819	7	65884166
ARS-BFGL-NGS-114212	8	46239174
ARS-BFGL-NGS-99602	8	46271357
ARS-BFGL-NGS-2224	8	46299075
BTB-01535731	8	46369566
BTB-01535697	8	46409283
BTB-01535659	8	46468733
ARS-BFGL-NGS-80030	8	46577889
ARS-BFGL-NGS-103766	8	46729375
Hapmap25854-BTA-149442	8	46826308
UA-IFASA-1863	8	46877924
ARS-BFGL-NGS-64295	11	41647520
Hapmap24893-BTA-27904	11	41740456
ARS-BFGL-NGS-10028	11	41789973
Hapmap54844-rs29022274	11	41833490
ARS-BFGL-BAC-12994	11	41854251
BTA-93062-no-rs	11	41992022
Hapmap39698-BTA-93047	11	42070693
BTA-99919-no-rs	11	42102223
BTA-99924-no-rs	11	42133104
ARS-BFGL-NGS-24591	11	42330073
ARS-BFGL-NGS-1097	19	34836416
ARS-BFGL-NGS-82204	19	35003592
ARS-BFGL-NGS-118018	19	35061702
ARS-BFGL-NGS-68563	19	35102613
ARS-BFGL-NGS-109844	19	35167831
ARS-BFGL-NGS-101953	19	35191657
ARS-BFGL-NGS-111809	19	35230173
ARS-BFGL-NGS-4759	19	35253851
ARS-BFGL-NGS-23022	19	35286010
ARS-BFGL-NGS-100460	19	35308991
ARS-BFGL-NGS-56555	20	6477168
BTB-00252636	20	6505111

ARS-BFGL-NGS-4292	20	6534735
BTB-00769424	20	6605865
BTB-00769318	20	6638385
ARS-BFGL-BAC-32398	20	6699756
Hapmap38898-BTA-51152	20	6736039
ARS-BFGL-NGS-108897	20	6832903
ARS-BFGL-NGS-20134	20	6912492
ARS-BFGL-NGS-54249	20	6935534
BTB-00119427	27	22757505
ARS-BFGL-NGS-11088	27	22779747
ARS-BFGL-NGS-116604	27	22811813
Hapmap44556-BTA-66734	27	22839206
BTB-00119229	27	22922395
BTB-00119211	27	22936249
ARS-BFGL-NGS-113093	27	22980780
Hapmap39675-BTA-66683	27	23040097
ARS-BFGL-NGS-5143	27	23104834
ARS-BFGL-NGS-38238	27	23144459

Appendix H

Supplementary Table 5.4. SNPs (60) within the six significant windows for 13 dpm collagen solubility.

SNPID	Chromosome	Position (bp)
BTA-71558-no-rs	4	81231390
BTA-71564-no-rs	4	81255320
Hapmap40292-BTA-71565	4	81400732
BTB-00201173	4	81433898
BTA-71570-no-rs	4	81497187
Hapmap41792-BTA-18792	4	81624828
BTA-71575-no-rs	4	81678235
Hapmap60835-rs29021024	4	81733900
ARS-BFGL-NGS-90094	4	81789767
BTA-87133-no-rs	4	81830858
BTA-112689-no-rs	6	12447020
ARS-BFGL-NGS-109709	6	12483908
BTB-01711686	6	12521271
BTA-88018-no-rs	6	12558447
Hapmap26355-BTA-123034	6	12602914
Hapmap42836-BTA-120227	6	12627595
BTB-01968603	6	12648459
Hapmap50605-BTA-16738	6	12669044
Hapmap59328-rs29016355	6	12703601
ARS-BFGL-NGS-83765	6	12741496
BTB-01945504	7	37839380
BTA-90692-no-rs	7	37865409
BTB-01414346	7	37908535
Hapmap49758-BTA-78793	7	37952883
BTB-00304608	7	37983978
Hapmap54492-rs29018585	7	38023859
BTB-00304702	7	38046316
Hapmap58358-rs29011315	7	38075277
UA-IFASA-2263	7	38123606
BTB-00305112	7	38196134
ARS-BFGL-NGS-107641	11	12513910
ARS-BFGL-NGS-13182	11	12561278
ARS-BFGL-NGS-21642	11	12596662
BTA-85474-no-rs	11	12668083
ARS-BFGL-NGS-37149	11	12717282
ARS-BFGL-NGS-100878	11	12856120
BTA-85470-no-rs	11	12877885
BTB-00460506	11	12947549

Hapmap48102-BTA-85468	11	12970800
ARS-BFGL-NGS-20431	11	13090203
ARS-BFGL-NGS-75368	14	79715760
ARS-BFGL-NGS-116056	14	79751498
ARS-BFGL-NGS-42962	14	79800617
ARS-BFGL-NGS-27929	14	79834107
ARS-BFGL-NGS-106221	14	79929279
ARS-BFGL-BAC-19843	14	79952134
UA-IFASA-9372	14	79972473
UA-IFASA-5839	14	80010342
Hapmap56994-rs29010282	14	80082712
ARS-USMARC-Parent-DQ846692-rs29010281	14	80082923
BTB-00660988	16	73711818
ARS-BFGL-NGS-36880	16	73736551
ARS-BFGL-NGS-84624	16	73801988
Hapmap47939-BTA-39867	16	73840001
ARS-BFGL-NGS-64329	16	73874593
Hapmap55487-rs29023215	16	73898020
ARS-BFGL-NGS-106233	16	73923140
BTB-00661933	16	74003832
Hapmap51565-BTA-122868	16	74046418
ARS-BFGL-NGS-15423	16	74158269

Appendix I

Supplementary Table 5.5. Nearby RefSeq genes (64) of significant windows for 3 dpm total collagen.

Gene name	Gene stable ID	Gene type	Gene start (bp)	Gene end (bp)
ABHD8	ENSBTAG00000025809	protein_coding	5822739	5829866
AK3	ENSBTAG00000017147	protein_coding	39797196	39819493
ANKLE1	ENSBTAG00000012592	protein_coding	5830530	5836468
ANO8	ENSBTAG00000017074	protein_coding	5794556	5803719
BABAM1	ENSBTAG00000012587	protein_coding	5837516	5843414
bta-mir-12018	ENSBTAG00000054611	miRNA	22962266	22962323
bta-mir-204	ENSBTAG00000029862	miRNA	46962508	46962613
bta-mir-2285i	ENSBTAG00000047378	miRNA	46642180	46642257
bta-mir-2470	ENSBTAG00000045064	miRNA	40620793	40620866
bta-mir-2471	ENSBTAG00000045466	miRNA	40311803	40311872
bta-mir-8550	ENSBTAG00000051297	miRNA	6134419	6134475
C19H17orf80	ENSBTAG00000006089	protein_coding	58084841	58095624
CDC37L1	ENSBTAG00000017137	protein_coding	39822427	39841618
CDC42EP4	ENSBTAG00000053044	protein_coding	58037532	58058085
CDC42SE2	ENSBTAG00000005961	protein_coding	23073268	23192277
COG1	ENSBTAG00000006087	protein_coding	58115492	58127648
COLGALT1	ENSBTAG00000012678	protein_coding	5543272	5566733
CPAMD8	ENSBTAG00000009331	protein_coding	6073318	6174087
DDA1	ENSBTAG00000017068	protein_coding	5807201	5817259
F2RL3	ENSBTAG00000033278	protein_coding	6175406	6177619
FAM104A	ENSBTAG00000006088	protein_coding	58095733	58114837
FAM129C	ENSBTAG00000018946	protein_coding	5568730	5586773
FCHO1	ENSBTAG00000002136	protein_coding	5368410	5395426
FNIP1	ENSBTAG00000025443	protein_coding	22699064	22818191
GCNT1	ENSBTAG00000012757	protein_coding	52522641	52568603
GTPBP3	ENSBTAG00000004200	protein_coding	5787153	5791859
HAUS8	ENSBTAG00000007366	protein_coding	5991339	6016463
HINT1	ENSBTAG00000010959	protein_coding	23271324	23278556
KLF9	ENSBTAG00000016229	protein_coding	46586710	46611498
LYRM7	ENSBTAG00000010961	protein_coding	23244080	23265677
MAP1S	ENSBTAG00000020709	protein_coding	5408672	5440703
MIR101-2	ENSBTAG00000029932	miRNA	39689023	39689113
MRPL34	ENSBTAG00000014579	protein_coding	5819753	5821004
MVB12A	ENSBTAG00000003914	protein_coding	5683952	5688015
MYO9B	ENSBTAG00000011125	protein_coding	5883221	5969910
NR2F6	ENSBTAG00000011136	protein_coding	5859425	5870048
NWD1	ENSBTAG00000009328	protein_coding	6235790	6318228
NXNL1	ENSBTAG00000016773	protein_coding	5658324	5663017

OCEL1	ENSBTAG00000011135	protein_coding	5872496	5874573
PCSK5	ENSBTAG00000008101	protein_coding	51911490	52419336
PGLS	ENSBTAG00000016783	protein_coding	5590439	5599169
PLPP6	ENSBTAG00000011050	protein_coding	39863787	39864933
PLVAP	ENSBTAG00000005434	protein_coding	5762752	5781737
PRUNE2	ENSBTAG00000012991	protein_coding	52760745	52887222
RCL1	ENSBTAG00000018667	protein_coding	39548658	39743532
RF00001	ENSBTAG00000045468	rRNA	5844183	5844316
RF00015	ENSBTAG00000045163	snRNA	39700730	39700859
RF00026	ENSBTAG00000045408	snRNA	80423119	80423224
RF00100	ENSBTAG00000047075	misc_RNA	57868356	57868692
RF01894	ENSBTAG00000053573	misc_RNA	52842493	52842902
RF02271	ENSBTAG00000053149	misc_RNA	23040128	23040297
RFK	ENSBTAG00000019345	protein_coding	52453482	52459253
SDK2	ENSBTAG00000044195	protein_coding	57874966	58018225
SIN3B	ENSBTAG00000009330	protein_coding	6181303	6224656
SLC1A1	ENSBTAG00000019125	protein_coding	39928081	40003010
SLC27A1	ENSBTAG00000016775	protein_coding	5605375	5646428
SLC39A11	ENSBTAG00000018837	protein_coding	58209287	58503188
SPATA6L	ENSBTAG00000011048	protein_coding	39841411	39915201
SSTR2	ENSBTAG00000017136	protein_coding	58153872	58154978
TMEM38A	ENSBTAG00000013614	protein_coding	6356455	6383026
TRPM3	ENSBTAG00000007778	protein_coding	46715833	46817682
UNC13A	ENSBTAG00000012682	protein_coding	5459776	5525065
USE1	ENSBTAG00000011134	protein_coding	5878528	5882165
USHBP1	ENSBTAG00000015108	protein_coding	5846576	5855842

Appendix J

Supplementary Table 5.6. Nearby RefSeq genes (128) of significant windows for 3 dpm collagen solubiltiy.

Gene name	Gene stable ID	Gene type	Gene start (bp)	Gene end (bp)
AATK	ENSBTAG00000019049	protein_coding	51538083	51576183
ACTG1	ENSBTAG00000006189	protein_coding	51259300	51262303
AFF3	ENSBTAG00000012449	protein_coding	4665771	5289303
AK8	ENSBTAG00000004092	protein_coding	102774910	102911331
ALYREF	ENSBTAG00000008498	protein_coding	50999623	51004298
ANAPC11	ENSBTAG00000001937	protein_coding	50994951	51000136
AP2B1	ENSBTAG00000020316	protein_coding	14648905	14757836
AQP2	ENSBTAG00000008374	protein_coding	29926245	29931719
AQP5	ENSBTAG00000026813	protein_coding	29897816	29914225
ARHGDIA	ENSBTAG00000030209	protein_coding	51024277	51028502
ARL16	ENSBTAG00000053129	protein_coding	51137131	51139166
ASIC1	ENSBTAG00000000970	protein_coding	29812653	29835158
ASPSCR1	ENSBTAG00000004632	protein_coding	50892622	50920822
BAHCC1	ENSBTAG00000016776	protein_coding	51302716	51357264
BAIAP2	ENSBTAG00000019044	protein_coding	51578606	51642239
BARHL1	ENSBTAG00000023801	protein_coding	102648411	102655723
BCDIN3D	ENSBTAG00000017781	protein_coding	30042064	30047717
bta-mir-2331	ENSBTAG00000045174	miRNA	14988931	14989001
bta-mir-2346	ENSBTAG00000044643	miRNA	50907254	50907332
bta-mir-2347	ENSBTAG00000053746	miRNA	50984772	50984830
bta-mir-2425	ENSBTAG00000045023	miRNA	30082250	30082330
bta-mir-338	ENSBTAG00000029775	miRNA	51569617	51569708
bta-mir-3533	ENSBTAG00000047677	miRNA	51261145	51261228
C19H17orf50	ENSBTAG00000045621	protein_coding	14614634	14615948
C1QL4	ENSBTAG00000032567	protein_coding	30485107	30487859
CCDC137	ENSBTAG00000000356	protein_coding	51148536	51154185
CCDC14	ENSBTAG00000000566	protein_coding	68289450	68332524
CCL14	ENSBTAG00000010738	protein_coding	14460812	14465640
CCL16	ENSBTAG00000053578	protein_coding	14468439	14475253
CCL3	ENSBTAG00000025250	protein_coding	14243466	14362161
CCL4	ENSBTAG00000025257	protein_coding	14339700	14341265
CCL5	ENSBTAG00000053649	protein_coding	14508970	14516896
CCT6B	ENSBTAG00000020338	protein_coding	15120579	15156414
CEL	ENSBTAG00000007486	protein_coding	103058904	103067774
CENPX	ENSBTAG00000004108	protein_coding	50887829	50891455
CEP131	ENSBTAG00000000875	protein_coding	51500747	51517013
CERS5	ENSBTAG00000017395	protein_coding	29736126	29766044
CFAP77	ENSBTAG00000019254	protein_coding	102485125	102638859

CHMP6	ENSBTAG00000038745	protein_coding	51669524	51676812
CHST10	ENSBTAG00000004328	protein_coding	5501359	5522717
COX14	ENSBTAG00000000808	protein_coding	29782810	29787103
DCXR	ENSBTAG00000047043	protein_coding	50854405	50856944
DDX31	ENSBTAG00000003530	protein_coding	102659360	102733192
DNAJC22	ENSBTAG00000016398	protein_coding	30470981	30474172
FAAP100	ENSBTAG00000019104	protein_coding	51224646	51233871
FAIM2	ENSBTAG00000017504	protein_coding	29983972	30018827
FAM186A	ENSBTAG00000023546	protein_coding	29562885	29584566
FAM186B	ENSBTAG00000019283	protein_coding	30233506	30249541
FMNL3	ENSBTAG00000016593	protein_coding	30151294	30202352
FNDC8	ENSBTAG00000019092	protein_coding	14964939	14976767
FSCN2	ENSBTAG00000024932	protein_coding	51236231	51242016
GAS2L2	ENSBTAG00000018519	protein_coding	14621338	14628732
GBGT1	ENSBTAG00000030319	protein_coding	103136831	103147276
GCGR	ENSBTAG00000003786	protein_coding	51063960	51072587
GCNT1	ENSBTAG00000012757	protein_coding	52522641	52568603
GFI1B	ENSBTAG00000012772	protein_coding	103007273	103012074
GLT6D1	ENSBTAG00000020249	protein_coding	103271493	103279630
GPD1	ENSBTAG00000016296	protein_coding	29788762	29795178
GTF3C4	ENSBTAG00000004091	protein_coding	102733925	102751664
HEATR9	ENSBTAG00000002850	protein_coding	14519263	14535861
HGS	ENSBTAG00000000411	protein_coding	51123898	51137090
KALRN	ENSBTAG00000002640	protein_coding	68647206	68942661
KCNH3	ENSBTAG00000019277	protein_coding	30274565	30293396
KCNT1	ENSBTAG00000018975	protein_coding	103335988	103387085
LCN9	ENSBTAG00000038972	protein_coding	103281334	103283945
LIG3	ENSBTAG00000018689	protein_coding	15077781	15098380
LIMA1	ENSBTAG00000012342	protein_coding	29637176	29729199
LRRC45	ENSBTAG00000004111	protein_coding	50879326	50887717
MAFG	ENSBTAG00000000040	protein_coding	50967339	50972885
MCRIP1	ENSBTAG00000019812	protein_coding	51050656	51058641
MCRS1	ENSBTAG00000019281	protein_coding	30264979	30274823
MMP28	ENSBTAG00000006086	protein_coding	14587761	14613432
MRPL12	ENSBTAG00000000417	protein_coding	51118070	51122495
MYADML2	ENSBTAG00000000044	protein_coding	50953006	50956142
MYLK	ENSBTAG00000014567	protein_coding	67982278	68068472
NCKAP5L	ENSBTAG00000004851	protein_coding	30062441	30093874
NDUFAF8	ENSBTAG00000053886	protein_coding	51480485	51483148
NLE1	ENSBTAG00000019094	protein_coding	14954563	14963358
NMS	ENSBTAG00000034184	protein_coding	5537564	5547779
NOTUM	ENSBTAG00000053558	protein_coding	50931392	50942268
NOVA1	ENSBTAG00000002170	protein_coding	36714178	36879180
NPB	ENSBTAG00000013898	protein_coding	50992167	50992784
NPLOC4	ENSBTAG00000019105	protein_coding	51171780	51221977

OXLD1	ENSBTAG00000000355	protein_coding	51154298	51156611
P4HB	ENSBTAG00000006045	protein_coding	51034066	51044560
PAEP	ENSBTAG00000014678	protein_coding	103255824	103264276
PCSK5	ENSBTAG00000008101	protein_coding	51911490	52419336
PCYT2	ENSBTAG00000001868	protein_coding	50982926	50990089
PDE6G	ENSBTAG00000000354	protein_coding	51158511	51160605
PEX12	ENSBTAG00000019723	protein_coding	14763783	14768000
PPP1R27	ENSBTAG00000024929	protein_coding	51048368	51050321
PRPF40B	ENSBTAG00000016589	protein_coding	30202309	30240990
PRPH	ENSBTAG00000017864	protein_coding	30519199	30522802
PRUNE2	ENSBTAG00000012991	protein_coding	52760745	52887222
PYCR1	ENSBTAG00000000042	protein_coding	50957969	50962684
RAC3	ENSBTAG00000022927	protein_coding	50863256	50879273
RACGAP1	ENSBTAG00000012784	protein_coding	29851713	29890966
RAD51D	ENSBTAG00000019082	protein_coding	14978195	14991205
RALGDS	ENSBTAG00000004194	protein_coding	103082834	103132073
RASL10B	ENSBTAG00000018518	protein_coding	14632048	14641729
REV1	ENSBTAG00000043999	protein_coding	4533386	4616248
RF00003	ENSBTAG00000028421	snRNA	14874860	14875020
RF00015	ENSBTAG00000044700	snRNA	68361091	68361241
RF00026	ENSBTAG00000052595	snRNA	29621280	29621386
RF00100	ENSBTAG00000043745	misc_RNA	51380231	51380555
RF00288	ENSBTAG00000042305	snoRNA	14769240	14769336
RFFL	ENSBTAG00000013645	protein_coding	14997717	15072713
RFK	ENSBTAG00000019345	protein_coding	52453482	52459253
ROPN1	ENSBTAG00000006947	protein_coding	68349806	68384919
RPTOR	ENSBTAG00000002883	protein_coding	51687441	52010821
SETX	ENSBTAG00000024822	protein_coding	102338051	102426270
SIRT7	ENSBTAG00000000039	protein_coding	50976407	50982602
SLC38A10	ENSBTAG00000018271	protein_coding	51437111	51478799
SLFN11	ENSBTAG00000019437	protein_coding	14810212	14822401
SLFN14	ENSBTAG00000039206	protein_coding	14780282	14789087
SMARCD1	ENSBTAG00000037935	protein_coding	29799573	29810207
SPACA9	ENSBTAG00000005189	protein_coding	102911371	102922553
SPATS2	ENSBTAG00000004660	protein_coding	30304006	30378369
TAF15	ENSBTAG00000006916	protein_coding	14543295	14573019
TEPSIN	ENSBTAG00000019972	protein_coding	51483169	51493993
TMBIM6	ENSBTAG00000018588	protein_coding	30112133	30128722
TROAP	ENSBTAG00000008499	protein_coding	30487010	30495995
TSC1	ENSBTAG00000005190	protein_coding	102926937	102960628
TSPAN10	ENSBTAG00000040573	protein_coding	51161792	51165910
TTF1	ENSBTAG00000018710	protein_coding	102451053	102480455
UNC45B	ENSBTAG00000002898	protein_coding	14913862	14947268
WFDC18	ENSBTAG00000039504	protein_coding	14300710	14302662

ZNF830	ENSBTAG00000020340	protein_coding	15118728	15120467

Appendix K

Supplementary Table 5.7. Nearby RefSeq genes (144) of significant windows for 13 dpm total collagen.

Gene name	Gene stable ID	Gene type	Gene start (bp)	Gene end (bp)
ACER1	ENSBTAG00000008095	protein_coding	18105651	18128839
ACSBG2	ENSBTAG00000009105	protein_coding	18256064	18269611
ADAMTS4	ENSBTAG00000013210	protein_coding	8303389	8311838
ALKBH5	ENSBTAG00000025046	protein_coding	34422370	34439576
APBA1	ENSBTAG00000008877	protein_coding	45576614	45814041
APOA2	ENSBTAG00000009212	protein_coding	8282869	8284122
ARHGAP30	ENSBTAG00000017875	protein_coding	8420191	8435163
ASAP3	ENSBTAG00000012263	protein_coding	129367152	129417868
ATPAF2	ENSBTAG00000008801	protein_coding	34524223	34536057
B4GALT3	ENSBTAG00000021897	protein_coding	8321014	8326857
bta-mir-11983	ENSBTAG00000048768	miRNA	8811960	8812023
bta-mir-2285ah	ENSBTAG00000054842	miRNA	65993836	65993914
bta-mir-2285i	ENSBTAG00000047378	miRNA	46642180	46642257
bta-mir-33b	ENSBTAG00000036437	miRNA	34647548	34647641
bta-mir-3432b	ENSBTAG00000045856	miRNA	17838214	17838310
bta-mir-584-6	ENSBTAG00000030064	miRNA	6324092	6324167
bta-mir-6535	ENSBTAG00000055025	miRNA	17755619	17755691
C1QA	ENSBTAG00000007153	protein_coding	130189849	130193588
C1QB	ENSBTAG00000011196	protein_coding	130166509	130173608
C1QC	ENSBTAG00000011193	protein_coding	130181311	130185682
C3	ENSBTAG00000017280	protein_coding	17773675	17810616
C8H9orf135	ENSBTAG00000033396	protein_coding	45949379	46053409
CAPS	ENSBTAG00000006069	protein_coding	18466099	18467633
CATSPERD	ENSBTAG00000001796	protein_coding	18552769	18589512
CD244	ENSBTAG00000002951	protein_coding	8695315	8755426
CD48	ENSBTAG00000011238	protein_coding	8901877	8932543
CD70	ENSBTAG00000009752	protein_coding	17903865	17908687
CD84	ENSBTAG00000019033	protein_coding	9076733	9119524
CLPP	ENSBTAG00000014712	protein_coding	18077043	18084803
COPS3	ENSBTAG00000018973	protein_coding	34886735	34905324
CRB3	ENSBTAG00000051530	protein_coding	18006370	18008564
DEDD	ENSBTAG00000020154	protein_coding	8363117	8373311
DENND1C	ENSBTAG00000031809	protein_coding	17996263	18005955
DHRS7B	ENSBTAG00000010559	protein_coding	35165031	35212185
DRC3	ENSBTAG00000008798	protein_coding	34536462	34556509
DRG2	ENSBTAG00000006517	protein_coding	34497627	34506489
DUS3L	ENSBTAG00000011842	protein_coding	18541354	18546361
E2F2	ENSBTAG00000014400	protein_coding	129326862	129343702

ENC1	ENSBTAG00000026369	protein_coding	6918477	6930788
EPHA8	ENSBTAG00000020102	protein_coding	130225773	130254992
EPHB2	ENSBTAG00000045902	protein_coding	129898024	130038309
F11R	ENSBTAG00000017846	protein_coding	8464412	8488977
FAM114A2	ENSBTAG00000017078	protein_coding	65295989	65330574
FAM169A	ENSBTAG00000016002	protein_coding	6676797	6738543
FCER1G	ENSBTAG00000024503	protein_coding	8286937	8290169
FLCN	ENSBTAG00000008010	protein_coding	34915672	34932182
FLII	ENSBTAG00000016161	protein_coding	34386823	34398726
FUT6	ENSBTAG00000000414	protein_coding	18515026	18516457
GALNT10	ENSBTAG00000034113	protein_coding	65477934	65700088
GFM2	ENSBTAG00000015519	protein_coding	6750176	6801800
GID4	ENSBTAG00000008802	protein_coding	34509785	34524187
GLS	ENSBTAG00000007863	protein_coding	79427772	79481911
GPR108	ENSBTAG00000017291	protein_coding	17759546	17767568
GRIA1	ENSBTAG00000005800	protein_coding	64771807	65118334
GTF2F1	ENSBTAG00000021016	protein_coding	18063616	18069587
GYPC	ENSBTAG00000014863	protein_coding	78885394	78934052
HAND1	ENSBTAG00000002335	protein_coding	65748838	65752153
HEXB	ENSBTAG00000015512	protein_coding	6801767	6834107
HNRNPR	ENSBTAG00000016578	protein_coding	129500051	129531646
HSD11B1L	ENSBTAG00000001793	protein_coding	18611628	18616460
HTR1D	ENSBTAG00000040329	protein_coding	129644896	129646029
ID3	ENSBTAG00000030425	protein_coding	129296772	129298371
ITLN2	ENSBTAG00000048662	protein_coding	8591925	8602122
KCNJ12	ENSBTAG00000020061	protein_coding	35328653	35363115
KDM1A	ENSBTAG00000009500	protein_coding	129770258	129836290
KHSRP	ENSBTAG00000021018	protein_coding	18035388	18047821
KLF9	ENSBTAG00000016229	protein_coding	46586710	46611498
KLHDC9	ENSBTAG00000020150	protein_coding	8389186	8391006
LACTBL1	ENSBTAG00000030413	protein_coding	129863541	129878003
LARP1	ENSBTAG00000008443	protein_coding	65952521	66013847
LLGL1	ENSBTAG00000004635	protein_coding	34398896	34413732
LONP1	ENSBTAG00000001795	protein_coding	18590319	18608816
LUZP1	ENSBTAG00000009502	protein_coding	129672631	129764693
LY9	ENSBTAG00000002947	protein_coding	8760842	8788290
MAMDC2	ENSBTAG00000017369	protein_coding	46233994	46396740
MAP2K3	ENSBTAG00000010576	protein_coding	35255751	35273731
MED9	ENSBTAG00000020517	protein_coding	34822995	34828048
MFAP3	ENSBTAG00000017079	protein_coding	65340082	65347501
MICOS13	ENSBTAG00000001792	protein_coding	18617143	18618572
MIEF2	ENSBTAG00000017081	protein_coding	34382621	34386321
MLLT1	ENSBTAG00000002277	protein_coding	18141671	18210109
MPRIP	ENSBTAG00000010534	protein_coding	34949651	35042578
MSX2	ENSBTAG00000013873	protein_coding	6444184	6449312

MYO1B	ENSBTAG00000011256	protein_coding	79795528	79994785
NDUFA11	ENSBTAG00000019025	protein_coding	18473539	18479129
NDUFS2	ENSBTAG00000002203	protein_coding	8290744	8300810
NECTIN4	ENSBTAG00000017877	protein_coding	8403138	8419412
NIT1	ENSBTAG00000020153	protein_coding	8373204	8376843
NR1I3	ENSBTAG00000009215	protein_coding	8271660	8276977
NRTN	ENSBTAG00000000413	protein_coding	18518580	18532314
NSA2	ENSBTAG00000003066	protein_coding	6742915	6750047
NT5M	ENSBTAG00000030820	protein_coding	34870103	34882343
PCP4L1	ENSBTAG00000040512	protein_coding	8231447	8258195
PEMT	ENSBTAG00000016432	protein_coding	34780764	34816442
PFDN2	ENSBTAG00000020152	protein_coding	8376916	8388862
PLD6	ENSBTAG00000008009	protein_coding	34939346	34942156
PPOX	ENSBTAG00000021894	protein_coding	8326933	8330785
PRR22	ENSBTAG00000025550	protein_coding	18545676	18548664
PSPN	ENSBTAG00000031794	protein_coding	18073040	18073585
PTAR1	ENSBTAG00000005826	protein_coding	45852527	45894473
RAI1	ENSBTAG00000004768	protein_coding	34650992	34752887
RANBP3	ENSBTAG00000006070	protein_coding	18414908	18464857
RASD1	ENSBTAG00000020520	protein_coding	34819717	34821443
RF00001	ENSBTAG00000043920	rRNA	22680010	22680110
RF00026	ENSBTAG00000048702	snRNA	129387213	129387319
RF00493	ENSBTAG00000042910	snoRNA	18550778	18550847
RF00494	ENSBTAG00000042283	snoRNA	18550534	18550613
RF00612	ENSBTAG00000044835	snoRNA	78876480	78876535
RFX2	ENSBTAG00000017661	protein_coding	18305239	18398128
RPL36	ENSBTAG00000001794	protein_coding	18608950	18610219
SAFB	ENSBTAG00000007875	protein_coding	18621612	18651390
SAP30L	ENSBTAG00000004000	protein_coding	65722185	65730845
SH2D3A	ENSBTAG00000013857	protein_coding	17732309	17747008
SHMT1	ENSBTAG00000017094	protein_coding	34334364	34350038
SLAMF1	ENSBTAG00000007927	protein_coding	8969591	9008636
SLAMF6	ENSBTAG00000014368	protein_coding	9152844	9174085
SLAMF7	ENSBTAG00000001197	protein_coding	8848948	8864962
SLC25A23	ENSBTAG00000003491	protein_coding	18011743	18026173
SLC25A41	ENSBTAG00000020017	protein_coding	18027580	18034407
SMC5	ENSBTAG00000018437	protein_coding	46411542	46530522
SMCR8	ENSBTAG00000017090	protein_coding	34353294	34358390
SMIM15	ENSBTAG00000048033	protein_coding	65416423	65416647
SREBF1	ENSBTAG00000007884	protein_coding	34633133	34649213
STAT1	ENSBTAG00000007867	protein_coding	79518741	79560102
STAT4	ENSBTAG00000046699	protein_coding	79568840	79673336
TCEA3	ENSBTAG00000038865	protein_coding	129421761	129471183
TEX46	ENSBTAG00000053631	protein_coding	129843757	129846448
TMEM11	ENSBTAG00000010566	protein_coding	35212505	35235654

TNFRSF13B	ENSBTAG00000015298	protein_coding	35095243	35118131
TNFSF14	ENSBTAG00000012223	protein_coding	17839032	17843838
TNFSF9	ENSBTAG00000046266	protein_coding	17963471	17966413
TOM1L2	ENSBTAG00000003983	protein_coding	34556866	34625228
TOMM40L	ENSBTAG00000009213	protein_coding	8275799	8280141
TOP3A	ENSBTAG00000017087	protein_coding	34359100	34379578
TRIP10	ENSBTAG00000047424	protein_coding	17747332	17757129
TRPM3	ENSBTAG00000007778	protein_coding	46715833	46817682
TSTD1	ENSBTAG00000017856	protein_coding	8442910	8444112
TUBB4A	ENSBTAG00000021013	protein_coding	17981998	17988028
UFC1	ENSBTAG00000021705	protein_coding	8338252	8362436
USF1	ENSBTAG00000017873	protein_coding	8436890	8442549
USP21	ENSBTAG00000021893	protein_coding	8331489	8337654
VAV1	ENSBTAG00000039160	protein_coding	17664498	17728163
VMAC	ENSBTAG00000006067	protein_coding	18469286	18472439
ZNF436	ENSBTAG00000002594	protein_coding	129478393	129483457

Appendix L

Supplementary Table 5.8. Nearby RefSeq genes (36) of significant windows for 13 dpm collagen solubility.

Gene name	Gene stable ID	Gene type	Gene start (bp)	Gene end (bp)
ARL10	ENSBTAG00000025345	protein_coding	37840043	37846096
bta-mir-205	ENSBTAG00000029854	miRNA	73900707	73900775
C16H1orf74	ENSBTAG00000002848	protein_coding	73522276	73524532
CAMK1G	ENSBTAG00000016541	protein_coding	73713243	73744243
CAMK2D	ENSBTAG00000014463	protein_coding	11800357	12107600
CDHR2	ENSBTAG00000025340	protein_coding	38024335	38070254
CDK13	ENSBTAG00000001528	protein_coding	80952446	81075991
CLTB	ENSBTAG00000010740	protein_coding	37857326	37879421
COMMD10	ENSBTAG00000019071	protein_coding	37598772	37783067
CYP26B1	ENSBTAG00000012212	protein_coding	12376837	12396521
DYSF	ENSBTAG00000013290	protein_coding	12899573	13123869
EIF4E1B	ENSBTAG00000003103	protein_coding	38111499	38114298
EXOC6B	ENSBTAG00000020799	protein_coding	11625730	12344742
FAF2	ENSBTAG00000017744	protein_coding	37916685	37985670
GPRIN1	ENSBTAG00000044035	protein_coding	38070323	38073091
HIGD2A	ENSBTAG00000010735	protein_coding	37856220	37857202
HK3	ENSBTAG00000014898	protein_coding	38332119	38352118
HSD11B1	ENSBTAG00000015086	protein_coding	73564739	73634329
IRF6	ENSBTAG00000002849	protein_coding	73502081	73518531
LAMB3	ENSBTAG00000016542	protein_coding	73668603	73712367
MPLKIP	ENSBTAG00000018715	protein_coding	80929262	80931409
NOP16	ENSBTAG00000010734	protein_coding	37851533	37856390
POU6F2	ENSBTAG00000013648	protein_coding	81370877	81759991
RALA	ENSBTAG00000006661	protein_coding	81187581	81244481
RF00001	ENSBTAG00000044497	rRNA	73929727	73929836
RF00026	ENSBTAG00000051643	snRNA	12698870	12698976
RNF44	ENSBTAG00000017748	protein_coding	37999787	38005337
SEMA6A	ENSBTAG00000020489	protein_coding	37316829	37446102
SNCB	ENSBTAG00000009803	protein_coding	38091557	38100473
SUGCT	ENSBTAG00000032121	protein_coding	80159127	80928936
SYT14	ENSBTAG00000046723	protein_coding	73169607	73359804
TRAF3IP3	ENSBTAG00000002846	protein_coding	73524525	73550750
TSPAN17	ENSBTAG00000017451	protein_coding	38116057	38126424
UNC5A	ENSBTAG00000014897	protein_coding	38268362	38331961
UTP25	ENSBTAG00000020886	protein_coding	73447321	73474258
YAE1	ENSBTAG00000001892	protein_coding	81286079	81291402