Changes to collagen heat solubility with post mortem ageing of beef and validation of genetic markers for collagen characteristics

by

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Abstract

Consumer acceptability of meat is dependent on eating satisfaction, of which meat tenderness is a principal factor. While there has been much work done to improve meat quality, and grading systems are in place to evaluate meat and guarantee a superior eating experience, the characteristics of intramuscular connective tissue can still affect that experience. Intramuscular connective tissue limits the tenderness of beef that is cooked quickly due to it being principally composed of collagen, a protein which requires prolonged cooking times for its degradation. Additionally, the mature collagen cross-links pyridinoline and Ehrlich's Chromogen further stabilize the collagen structure, leading to decreased collagen heat solubility, and have previously been associated with a marked increase in Warner Bratzler Shear Force (WBSF), an objective measure of meat toughness. Post mortem ageing of meat has been shown to decrease toughness, as the activity of enzymes responsible for the degradation of intramuscular collagen continues after slaughter. However, the use of growth promotants has been shown to have an adverse effect on meat quality and may limit the benefit of post mortem aging, and the effects of growth promotants on collagen characteristics is not well understood.

In this thesis, collagen and other meat quality characteristics such as WBSF were analyzed for the *longissimus thoracis* muscle of 198 crossbred steers, the samples from which were aged 7 days prior to analysis. Of these animals, 149 steers were part of a steroid treatment to evaluate the effects of steroid use on meat quality and collagen characteristics. Cattle were implanted with 200 mg progesterone and 20 mg estradiol benzoate, and a terminal implant composed of 120 trenbolone acetate and 24 mg estradiol. One group received one backgrounding implant (n=16), a second group received two backgrounding implants (n=16), a third received two backgrounding implants and one terminal implant (n=16), a fourth group received one

backgrounding implant and a terminal implant (n=16), and a fifth group received only a terminal implant (n=38). There were 47 animals in the control group (n=47) that received no implants. One part of each sample was refrigerated and aged for an additional 14 days for a total of 21 days to evaluate the effects of aging on collagen solubility.

Results showed that steroid use was associated with reduced quality grade (P=0.01), improved yield grade (P<0.01), slower carcass cooling and pH decline, decreased carcass L* scores (lightness), and some increase in WBSF when multiple implants were used. However, there was no effect of steroids on collagen characteristics. There was some improvement of collagen solubility in the longissimus muscle if the carcass graded Canada Prime and had increased fat content, although collagen solubility decreased with animal age. WBSF was mainly associated with animal age and intramuscular fat content. There was no effect of aging on collagen solubility after 7 days post-mortem.

Previous studies have identified single nucleotide polymorphisms (SNPs) within genes affecting total collagen, collagen cross-link synthesis, and collagenase activity. A genome-wide association study was designed to identify SNPs associated with collagen solubility and total collagen in 286 steers, and mature collagen crosslinks in 149 steers. In total there were 5 SNP windows associated with collagen solubility prior to aging the samples, 8 SNP windows associated with solubility after aging, and 5 windows relating to total collagen content. Further, 4 SNP windows were associated with Ehrlich's Chromogen concentration and 10 windows were related to pyridinoline concentration. Functional analysis using Ingenuity Pathway Analysis (IPA) software identified genes within these SNP windows relating to cellular growth and proliferation, especially the proliferation of tumor cells and the metastasis of cancer. The observations suggest the possibility for marker-assisted genomic selection for collagen

characteristics in beef cattle, however due to the small sample size of the study, a larger number of cattle will be needed to validate these results.

Preface

This thesis is an original work by Meghan Markowsky. No part of this thesis has been previously published.

The nature of the experiments performed on the samples as part of this thesis were considered Category A: non-invasive, and as such did not require ethics approval as there was no animal handling involved in the work performed for this thesis. While information was acquired from the University of Guelph, the Agriculture and Agri-Food Canada facilities in Lacombe, Alberta, and from the University of Alberta from previous research, the quantification of total collagen, collagen solubility, and mature collagen crosslinks, as well as the evaluation of the effects of post-mortem aging on collagen solubility in beef, was performed on animal tissue at the University of Alberta.

Data collection involving collagen characteristics, as well as all data analysis in this work was performed by me, with the exception of collagen data for animals from a previous study that were incorporated into Study 3, which was collected by Dr. Huaigang Lei.

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Chapter 1: Introduction and Literature Review

1.1 Introduction

As the world population increases, the amount of food that is produced must increase with it to ensure sufficient food for all. Meat is a food product that is valued for its high protein content and is derived from the muscles of species such as cattle, chicken, pig, sheep, and different types of fish. The animals whose tissue is consumed are specifically raised with the intent to be slaughtered and divided into saleable cuts of meat for the consumer. Beef is one of the major sources of animal-derived protein, and is one of the most expensive (StatCan, 2021), making it a high value product. As a result, consumers of beef are concerned with its eating quality, and perceived eating quality can drive beef value (Lusk et al. 2001). Colour is the most important trait considered by consumers when purchasing beef (Troy and Kerry, 2010), but tenderness as determined by intramuscular collagen content is what limits the value of much of the beef carcass (Bruce and Roy, 2018). Collagen is the main protein found in mammalian connective tissues, and it plays a significant role in contributing to the toughness of beef muscle and determines the value of beef cuts (Bruce and Roy, 2018). As connective tissue content varies between different muscles (Blanco et al. 2013), many high collagen content cuts are sold as ground beef rather than quick-cook steaks due to their relative toughness. By understanding the characteristics of collagen and how it influences meat tenderness, not only would the value of meat with higher collagen content be improved by limiting the negative effects collagen has on meat quality, but also there would be an increase in the consistency of meat quality for quick-cook steaks. Therefore, there is economic value in understanding how to minimize the contribution of collagen to meat toughness in beef products and ultimately improve the quality of the entire carcass.

Collagen, along with elastin in some muscles, is directly responsible for the 'background toughness' of beef, a term coined by Marsh and Leet (1966). The factors that affect collagen are numerous and need to be fully considered in any analysis that is undertaken to decrease the contribution of connective tissue to beef toughness. For example, the susceptibility of collagen to degrade into gelatin during cooking is related to the number of mature trivalent collagen crosslinks that stabilize the collagen structure (Girard et al. 2012; Roy et al. 2015). The formation of mature collagen crosslinks occurs with time, and in animals that are actively growing, such as younger animals, collagen synthesis would be higher, resulting in more soluble collagen than in mature animals (Purslow, 2018). Also, collagen is degraded by matrix metalloproteinases (MMPs) which are collagenases that are inhibited by tissue inhibitors of metalloproteinases (TIMPs), and so the stability of collagen can potentially change with post-mortem aging of beef as the structure is degraded and heat lability is increased. MMPs have been shown to cleave collagen during post-mortem ageing of lamb (Sylvestre et al. 2002), and proteolytic activity has been associated with increased tenderness in beef that has been aged for 29 days (Ekine-Dzivenu et al. 2017).

Recently, single nucleotide polymorphisms (SNPs) have been identified in the genes coding for collagen content in beef cattle (Sorbolini et al. 2017; Dunner et al. 2013), potentially allowing for the genetic selection of collagen traits in meat. The purpose of this review then is to understand the importance of beef quality, relate meat quality characteristics to the properties of connective tissue and collagen, and discuss genes that may influence collagen characteristics ante- and post-mortem that have the potential to be used to select for reduced contribution of connective tissue to beef toughness.

1.2 The importance of beef and beef quality

Beef is one of the most popular sources of animal protein in Canada. In 2017, the disappearance of beef as a source of animal protein was 25.17 kilograms per person, a rate of disappearance second only to that of chicken, which was consumed at a rate of 33.13 kg per person on average (StatCan, 2017). While the amount of beef that is consumed per person in Canada has decreased during recent years in comparison to chicken, beef remains an important source of protein.

The cost of producing beef is significant, as the cattle from which beef is derived must be housed, fed, and given medical care from certified veterinary professionals throughout their lives until they reach a marketable weight. There are then costs associated with transport, slaughter, and packaging of the meat once the animal has reached the desired size and age. With an increase in consumer concern for animal welfare (Ortega and Wolf, 2018), farmers face additional costs for being part of a certified humane program. To mitigate these costs, it is important that beef producers receive as much return on their investments as possible, and this is best achieved through producing high quality meat products. Indeed, consumers are willing to accept a price increase for meat if the quality of the meat, especially the tenderness, is guaranteed (Meyerding et al. 2018).

Meat quality is multifactorial, with physical factors such as juiciness, colour, springiness, drip loss, and tenderness all considered important (Holman et al. 2022; Khliji et al. 2010; Abdullah et al. 2014). However, meat tenderness is considered the most important characteristic associated with consumers accepting or rejecting meat (Jeremiah, 1982), and there has been research on improving the tenderness of beef as well as studying the factors that affect it (Zhao et al. 2012; Gonçalves et al. 2018). Many factors are now known to be involved in the formation of

beef tenderness including animal diet, production system, stress before slaughter, carcass temperature during early post-mortem ageing, the sex of the animal, and animal genetics (Duffy et al. 2017; Polkinghorne et al. 2018; Zhao et al. 2012; Hannula and Puolanne, 2004; Mberema et al. 2016; Dunner et al. 2013). These factors can affect proteolytic enzymes that degrade muscle proteins involved in meat toughness during post-mortem ageing (Bhat et al. 2018; Kemp et al. 2010). There is increasing interest in the role that collagen plays in "background toughness" of meat, particularly now that the effects of post-mortem proteolysis on myofibrillar proteins are well understood (Bhat et al. 2018). Examination of the enzymes involved in the breakdown of collagen post-mortem warrants review as these enzymes called matrix metalloproteinases (MMPs) most likely play a significant role in the contribution of collagen to aged beef toughness. MMPs continue to be active after meat is thawed following frozen storage (Souza-Tarla et al. 2005). To evaluate the effect of collagen and collagenases on beef tenderness, their role within living muscle must be considered, and their interaction with the structure of muscle tissue and the activity of muscle proteins in the living organism needs to be understood**.**

1.3 Muscle structure

There are three types of muscle in mammals, birds, and fish: smooth muscle, such as that involved in the the function and movement of the gastrointestinal tract; cardiac or heart muscle; and skeletal muscle. Although cardiac and smooth muscle are consumed in some countries, the muscle that is the focus of the North American meat production systems is skeletal muscle, and that will be the focus of this review. Skeletal muscle accounts for between 30% and 40% of a living organism (Lawrie, 1979) and it is composed of approximately 75% water, 20% protein, 1-10% fat, and 1% glycogen on average (Listrat et al. 2016). The ratio of these components varies between individuals, especially water and fat contents which tend to decrease and increase

respectively with animal age. The fat component can vary remarkably, from 1 to 10% (Listrat et al. 2016) and to even greater proportions in breeds such as Wagyu (Horii et al. 2009). Water is negatively correlated with fat (Franco et al. 2009), as fat contains very little water. Fat content also increases with age (Park et al. 2018), resulting in lower water content in the skeletal muscle of older animals.

Proteins in muscle are categorized into sarcoplasmic, myofibrillar, and connective tissue proteins (Lawrie, 1979). Sarcoplasmic proteins reside in the sarcoplasm of muscle, which is the cytoplasmic fluid in muscle fibers. These proteins are involved in many functions such as protein synthesis and degradation, electron transport, and there are hundreds of different sarcoplasmic proteins (Pearson and Young, 1989). Connective tissue proteins include collagen and elastin, which are extracellular and provide structural integrity to the muscle. Myofibrillar proteins constitute the sarcomere, which is the basic contractile unit of the muscle and facilitates movement.

Whole muscle is encased in a thick layer of connective tissue called the epimysium, which connects to the tendons and directly connects muscle to bone. Within muscle, bundles of muscle fibers are wrapped in a connective tissue called the perimysium and within these bundles, also known as fascicles, muscle cells are individually wrapped in another thin layer of connective tissue called the endomysium (Guo and Greaser, 2017). The diameter of these muscle fascicles can range from 1-5 mm and as animals age and their fascicles become larger, these are easily seen with the naked eye. The individual muscle fibres can be 20-80 μm in diameter, and are multinucleated (Purslow, 2017). In terms of meat toughness, muscles with numerous fascicles that are small in diameter tend to be tougher than muscles which contain mostly large

fascicles. MRI imaging of muscle fiber bundles can be used to predict meat tenderness alongside other methods of evaluation (Sifre et al. 2005).

The formation of muscle fibers occurs through a unique process called myogenesis, whereby somite cells during embryonic development differentiate into myoblasts, from which myocytes are derived. These myocytes then fuse to form the myotubes that make up muscle fibers (Feng et al. 2018). Not all muscle is produced by the same process however, with muscles of the head developing from the paraxial mesoderm rather than the lateral dermamyotome, from which the muscles of the limbs are developed (Francetic and Li, 2011). The number of muscle fibers within the animal is determined during fetal development and is fixed at birth, except in pigs where evidence has shown that there is some continued increase in fiber number up to 28 days postnatally (Bérard et al. 2011). In general, muscles grow postnatally only via hypertrophy of the muscle fibers. Satellite cells located on the surface of the muscle cell, between the sarcolemma and the basal lamina of the muscle cell (endomysium), donate nuclei to growing muscle fibers. Satellite cells multiply through division as they retain mitotic ability, and the daughter cells are either progenitor satellite cells, or cells which are dedicated to fusing with the muscle cell and providing a myonucleus (Dumont et al. 2015). Muscle cell hypertrophy also requires that the existing structures be altered to accommodate new myofibrils, therefore a balance between protein synthesis and protein degradation is necessary to allow for muscle growth (Purslow, 2017). In times of stress such as disease or starvation, muscles have been shown to atrophy, meaning the degradation of proteins exceeds protein synthesis (Bonaldo and Sandri, 2013).

The myofibrils are subunits of the multinucleate muscle cells and contain the actin and myosin myofilaments (Pearson and Young, 1989). Actin is a thin filamentous protein, while

myosin is a thick filament, and the inter-digitation of these proteins gives muscle a striated appearance when viewed microscopically. The interaction of actin and myosin allows for muscle movement in the presence of adenosine triphosphate (ATP), and in the absence of ATP this interaction is what contributes to the permanent bonding between actin and myosin, resulting in rigor mortis once ATP has been exhausted in the post-mortem muscle.

1.4 Myosin, actin, tropomyosin, and the troponin complex

Myosin comprises most of the thick filament within muscle that contributes to muscle contraction through interaction with actin. It is composed of two heavy chains and four light chains, where two light chains are associated with each heavy chain, and each heavy chain has a bulbous head that binds to ATP and actin (Purslow, 2017). As myosin is able to hydrolyze adenosine triphosphate into adenosine diphosphate and an independent phosphate ion, it is an ATPase, and it is this ATPase activity that drives muscular contraction.

Actin is the thin filament in muscle fibers. It is present as either globular or G-actin, and filamentous or F-actin. F-actin exists as a double helix of two actin strands wound around one another as seen in Figure 1.1 (Ecken et al. 2015). Wrapped around the actin protein is the troponin complex and tropomyosin, which regulate the binding of actin to myosin in muscle contraction through steric inhibition (Gordon et al. 2000). The troponin complex consists of troponin I (TnI), troponin T (TnT), and troponin C (TnC). When calcium is released from the sarcoplasmic reticulum, it binds to TnC, and this strengthens the bond between TnC and TnI. The strengthening of this bond weakens the bond between TnI and actin. These changes result in tropomyosin moving aside and exposing the myosin binding site on actin (Purslow, 2017), and the repeated interaction between the myosin and actin chains slides the chains along each other, which powers muscle contraction.

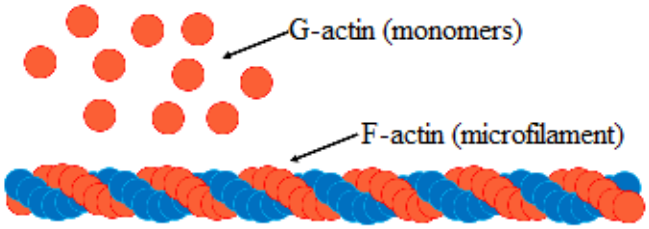

Figure 1.1: Single unit monomers of globular actin and polymer chains of filamentous f-actin

1.5 Muscle fiber types

Muscle fiber types are defined by multiple different but interrelated characteristics, including colour, metabolic or muscle fiber twitch function, and the protein isoform of the myosin heavy chain (Pearson and Young, 1989). Red muscle is considered “slow-twitch” muscle and uses an aerobic system to produce energy in the muscle, while white muscle is “fast-twitch” and uses an anaerobic, glycolytic system to produce energy (Shuman and Coughlin, 2018). Slow-twitch muscle is involved in sustained use and is more resistant to exhaustion, whereas fast-twitch muscle is used for short bursts of strength and tires quickly but can operate in a low oxygen environment.

Muscle fiber types have also been divided into types I, IIa, IIb, and IIx based on isoforms of the myosin heavy chains (Lefaucheur et al. 1998). Muscle fiber types that are defined by this system do not extend to the whole muscle, as all muscles contain varying combinations of the different fiber types, although muscles that are fatigue-resistant have an increased type I fiber content (Pearson and Young, 1989).

Finally, muscle fibers can be divided into the following types: slow-twitch oxidative, fast-twitch glycolytic, and fast-twitch oxidative/glycolytic, according to ATP production (Peter et al. 1972). This system is similar to the type I and II system of identification, where slow-

twitch fibers would be classified as type I fibers, and the fast-twitch fibers are both type II but are differentiated based on their oxidative capacity.

Type I muscle fibers seem to have a well-developed capillary network surrounding them, which may be due to the increased requirements for oxygen of this fiber type (Gotoh, 2003). As such, there tends to be increased intramuscular fat in muscles that have predominantly type I fibers as more fat can be transported through the bloodstream to the muscle through the capillary network. Type I muscle fiber content has also been associated with an increase in total collagen content in horse meat, and this may be due to type I fibers having a thicker endomysium layer (Roy et al. 2018).

Kim et al. (2018) examined the differences in quality characteristics of pork muscles in correlation with different fiber types. It was found that muscles with a high content of oxidative fibers had lower WBSF values and had a higher pH than those with a high proportion of glycolytic muscle fibers. These muscles also had a higher a* value, which is an objective measure of meat redness (Jacob, 2020). In the same study, muscles with a high content of glycolytic fibers were lighter in colour when raw, had an increased drip loss, and were tougher after cooking. These results are supported by the findings of Hwang et al. (2010) in which type I and type IIa muscle fiber content was positively associated with meat tenderness, whereas fast-twitch glycolytic type IIb fibers were negatively correlated with tenderness. These results suggest that muscles with greater slow-twitch oxidative fibers will be tender and may be darker and more red in colour.

1.6 Sarcomere

1.6.1 Sarcomere structure

The sarcomere is the smallest contractile unit of the muscle cell. It runs from Z-line to Z-line and repeats longitudinally along the length of skeletal muscle (Purslow, 2017). The

contraction and expansion of the sarcomeres within a muscle is what allows muscle movement. Sarcomeres have a striated appearance when viewed microscopically, and this is due to the alternating thick and thin filaments that make up the myofibril. Actinin is the key structural protein that composes the Z-disk, which anchors the actin filaments from sarcomere to sarcomere (Mukund and Subramaniam, 2019), while titin runs along the length of the thick filament and defines the length and organization of the myosin filaments (Linke, 2018). When muscles contract, the myosin heads move along the actin filaments. Desmin is another major protein that allows interaction of molecules and forms a chain of filaments, linking adjacent myofibrils (Mukund and Subramaniam, 2019). Mice with gene-knockout for desmin had weak and poorly configured muscles (Li et al. 1997), indicating that the protein connection between myofibrils was essential for coordinated movement and muscle strength.

1.6.2 Sarcomere length and meat tenderness

During rigor mortis, actin combines with myosin to form the actomyosin complex, an inextensible protein complex. This is due to the absence of ATP during post-mortem (Bendall, 1973), as hydrolysis of ATP is required to interrupt the bond between myosin and actin. During this process, sarcomere length has been shown to decrease, and this has been linked with increases in meat toughness. When placed in an ice bath and allowed to shorten, beef *semitendinosu*s muscle showed greater toughness than samples that were stretched and restrained prior to aging (Weaver et al. 2008). Indeed, when evaluating the tenderness of sun-dried beef, the sample with the greatest sarcomere length exhibited the lowest values for WBSF (and thus was more tender) and scored well when evaluated by a panel (Ishihara et al. 2013). There is also an interaction between sarcomere length and collagen softness. When sarcomere length is increased, connective tissue that is associated with the muscle has been found to be softer, and this has been

attributed to the increased tension placed on the connective tissue that is stretched over the sarcomere, which would have a lower resistance to an outside force (Aalhus et al. 1990).

The degree to which sarcomere shortening occurs is dependent largely on post-mortem pH and temperature. Cold shortening refers to sarcomere shortening as a result of the carcass being exposed to cold temperatures post-mortem while the muscle enters rigor, while in heat shortening the sarcomere length decreases as a result of prolonged exposure to high temperatures during the first 12 to 24 hours post-mortem. In a study reported by Locker and Hagyard (1963), the temperature at which minimal sarcomere shortening occurs was found to be between 14 and 19 ºC. In the case of heat shortening, which occurs at temperatures above 20 ºC, the cells enter rigor (deplete their energy stores) more quickly leaving less time for the sarcomeres to contract, which may explain why the reduction in sarcomere length is less than in cases of cold shortening (Ertbjerg and Puolanne, 2017). There is an interaction between pH and temperature on sarcomere length as well, with Hannula and Puolanne (2004) showing that a high pH produced tougher meat in samples at 7 ºC in comparison to samples with a lower pH, with the ideal pH being 5.7. This indicates that the effect of cold shortening is reduced when pH is low. However, only a certain degree of sarcomere shortening will contribute to meat toughness. Sarcomere shortening of less than 20% has been found to not have a significant effect on meat tenderness, therefore meat tenderness is not decided solely by the length of the sarcomeres in the muscle (Marsh and Leet, 1966).

1.7 Degradation by calpains, cathepsins and caspases

1.7.1 Calpains

Calpains are proteolytic enzymes, and they contribute to the degradation of muscle fibers post-mortem which contributes to the tenderization of meat (Bhat et al. 2018). Calpains are categorized into two types based on their respective concentration of calcium ions for activation:

milli-calpain (m-calpain) and micro-calpain (μ-calpain). Milli-calpain exhibit a far greater requirement for calcium than the μ-calpain to achieve activation, needing 400-800 μM of calcium for half-maximal proteolytic activity, while the μ-calpain in comparison require only 3-50 μM of calcium (Goll et al. 2003). A third calpain has been identified that is referred to as calpain-3; however, a study in calpain-3 gene knockout mice determined that calpain-3 is not involved in the post-mortem tenderization of meat (Geesink et al. 2005). Additionally, a review by Koohmaraie and Geesink (2006) found that m-calpain does not autolyze post-mortem, which means that the activity of m-calpain is maintained. As there is no difference in tenderization despite the continued activity of m-calpain, it is therefore considered to not be involved in the tenderization of meat during post-mortem ageing. Therefore, only μ-calpain seems to be involved in post-mortem tenderization of meat. In a study measuring beef toughness between heifers, steers and bulls, heifers that had tougher meat exhibited lower levels of μ-calpain specifically in comparison to heifers with more tender meat, which suggests that the difference in tenderness was due in large part to μ-calpain levels as the results were not confounded by differences in sex (Mberema et al. 2016).

Calpastatin is an inhibitor of m-calpain and μ-calpain, and thus limits the ability of calpains to contribute to muscle fibre degradation (Koohmaraie and Geesink, 2006). Again, in the study of Mberema et al. (2016) that compared calpain and calpastatin levels in bull, heifer and steer carcass muscles, the researchers found that steers had higher levels of calpastatin expression in comparison to bulls, although they had similar levels of μ-calpain expression. Meat from steers had significantly higher values for WBSF in comparison to bulls in this study. Heifers did have tougher meat than some steers, even though they had lower levels of calpastatin, however they also had lower μ-calpain expression than those steers. Tenderness

derived from calpain activity is therefore not dependant solely on the amount or activity of calpains or calpastatin, but on the interaction between the proteolytic enzyme and its inhibitor. Calpastatin also requires calcium ions to bind to calpains, but the requirement for half-maximal activity is lower than that of calpains (Otsuka and Goll, 1987). Calpains are intracellular enzymes that have no effect on collagen, but rather tenderize meat during the ageing process specifically through the degradation of myofibrillar proteins such as desmin and titin (Bhat et al. 2018). This indicates that although calpains do contribute to cooked meat toughness, it is through the contribution of myofibrillar proteins to meat toughness and not through the connective tissue portion.

1.7.2 Cathepsins

Cathepsins are proteolytic enzymes contained within the lysosome, of which there are multiple kinds identified including cathepsins B, D, E, F, K, L, and S. Cathepsins tend to operate at a reduced pH, usually between 3.0 and 6.5 (López-Bote 2017). To play a role in tenderization however, cathepsins must be released from the lysosome, and this may reduce the impact cathepsins have on meat tenderization if they are unable to escape the lysosome and access myofibril proteins (Hopkins and Thompson, 2002). Cathepsin B has been shown to successfully degrade cytoskeletal proteins despite having to be released from the lysosome, breaking down desmin by cleaving small dipeptide fragments starting at the C-terminal end of the protein (Baron et al. 2004). Tenderness in yak meat has also been associated with cathepsin B, H and L activity, with activity being low early post-mortem and increasing throughout the ageing process (Tian et al. 2013). Cathepsin activity can persist throughout post-mortem storage if permitted, such as in the process of dry-curing ham. While cathepsin D activity ended during the immediate post-mortem period, cathepsin B, H and L activity continued up to 15 months, albeit at a lowered

capacity compared to initial levels (Toldra et al. 1993). Cathepsin K has been shown to have some collagenase activity; however, this activity has not been linked to meat tenderness and is instead often evaluated in the context of disease proliferation in conditions such as osteoarthritis (Dejica et al. 2012). Also, cathepsin K cleaves collagen at the N-terminal region rather than at the typical collagenase cleavage site (Kafienah et al. 1998).

Cystatin is the inhibitor of cathepsins and has been associated with increased meat toughness. *Longissimus lumborum* muscle from crossbred Angus steers showed higher expression of cystatin mRNA in comparison to purebred Angus or Brangus steers, and exhibited increased meat toughness during mechanical tests and lower scores for tenderness when evaluated by a sensory panel (Gonzalez et al. 2014). As with calpains, the concentrations of cathepsins and cystatin, as well as the balance between their activity is likely to drive their contributions to cooked meat toughness, and that research remains to be conducted.

1.7.3 Caspases

Caspases play a role in cell apoptosis (programmed cell death) through the destruction of multiple intracellular structures that result in the cell's inability to survive (Earnshaw et al. 1999), though extracellular structures such as connective tissue are unaffected. Cell apoptosis is thought to be part of the meat tenderization process post-mortem (Sentandreu et al. 2002), although studies focusing on the relationship between caspase activity and tenderness are limited. Underwood et al. (2008) found no correlation between caspase activity post-mortem and WBSF values, and in addition there was no further activation of caspases, only a decrease in existing activity. However, a later study by Cao et al. (2013) found a positive association with caspase 3 activity and lower shear force values, and that caspase 3 was activated post-mortem by caspase 8 and 9 activity. It is unclear, then, the precise role that caspases may play in meat

tenderization but based on the findings of Cao and colleagues (2013), caspases may indicate the onset of tenderization without actually being responsible for the tenderization process themselves. Caspases are activated early in necrosis and would therefore be expected to be active in early post-mortem, which may indicate the onset of tenderization by early calpain activity, as these have been linked to the degradation of myofibrillar proteins, although this hypothesis remains to be tested.

1.8 Adipose tissue

Adipose tissue is located throughout the body, but it is primarily found in four locations in the body: subcutaneous fat, or fat under the skin, intramuscular and intermuscular fat, which is within the muscle and between muscles respectively, and visceral fat, which lies around the internal organs (Purslow, 2017). Adipose that is found in intramuscular connective tissue may play a role in meat tenderness. Adipose tissue comes in two forms, brown fat and white fat, and as brown fat is mostly found in cold weather adapted animals due to it being used for generation of heat (Cannon and Nedergaard, 2004), only white adipose tissue will be considered here.

Adipose tissue also plays a significant role in consumer acceptability of beef and overall meat value. Intramuscular adipose tissue, otherwise known as intramuscular fat (IMF) or marbling, contributes to increases in the tenderness of beef (Corbin et al. 2015). However, marbled beef with IMF content greater than 10% was also found to have a more intense flavor when evaluated by a trained sensory panel (Frank et al. 2017).

A study of horse meat (Roy et al. 2018) did not find a difference in IMF content between tough and tender cuts, but the IMF was deposited differently in the tender muscles. In tender cuts, the number of adipocytes within the perimysium was greater than in tough muscles and increased the thickness of the perimysium. These findings may inform how adipose tissue and

collagen networks interact to affect meat tenderness in beef, as previous studies have found that adipocytes can disrupt the collagen matrix, which may contribute to decreased toughness in beef, as the structural integrity of the muscle is compromised due to the alteration of the perimysium (Nishimura, 2015; Nishimura et al. 1999).

Differences in fat content can also lead to different measures of meat tenderness in cooked product. Puente et al. (2019) examined the differences in meat quality characteristics after aging between the four different quality grades of Canadian beef, these being Canada A, AA, AAA, and Prime in ascending order of marbling and IMF because quality grade is differentiated using visible intramuscular fat or marbling in the m. *longissimus thoracis* at the 12^{th}-13^{th} rib site of the beef carcass. These researchers found that *longissimus thoracis* muscle from carcasses graded Canada Prime and AAA were not only higher in intramuscular fat than carcasses graded Canada AA and A, but also that these carcasses had lower mean WBSF. This difference in meat tenderness was found after aging the samples for 14 days, as at 4 days there was no difference in tenderness between the four grades. This indicates that in beef cuts that respond to post-mortem aging, intramuscular fat content can be measured and used together with post-mortem aging to ensure cooked beef product of a consistently improved value. Canada AAA and Prime did not differ significantly in WBSF in this study, and as such Canada AAA *longissimus dorsi* steaks can be enjoyed by those consumers who are looking to reduce their fat intake without having to decrease the quality of their eating experience.

The inverse relationship between intramuscular fat and toughness is well established in muscles with low levels of collagen, such as the *longissimus thoracis et lumborum.* This is also the case in muscles with an increased amount of collagen, such as the *semitendinosus* muscle, which was found to have the highest total collagen content when compared to the *longissimus*

muscle and the *Triceps brachii* (Blanco et al. 2013). Kuber et al. (2004) examined the effects of a number of factors on meat tenderness and found that steaks from Wagyu animals had lower Warner-Bratzler shear force values than those of purebred Limousin animals, while also having higher quality grades and marbling scores.

1.9 Collagen

1.9.1 Connective tissue

Of the three strata of connective tissue which are epimysium, perimysium and endomysium, perimysium and endomysium are considered intramuscular connective tissue (IMCT). The amount of connective tissue varies from muscle to muscle (Purslow, 2005) due to differences in muscle activity and function. This is seen especially in a study of 14 different bovine muscles, where the endomysial collagen content of muscles on a dry matter basis ranged from 0.47% to 1.2%, and perimysial collagen content on a dry matter basis ranged from 0.43% to 4.6% (Purslow, 1999).

Collagen, the primary protein of connective tissue, is composed of three α-chains tightly wound in a triple helix. About 28 different types of collagens have been discovered (Richard-Blum, 2011), with each type performing different functions in the body. The main collagen types found in skeletal muscle are types I, III, IV, V and VI (Listrat et al. 2000). Of these, fibril forming collagen types I and III dominate, with the perimysium appearing to be composed mostly of collagen type I, and both type I and type III collagen being present in large amounts in the endomysium (Light and Champion, 1984).

Most collagen types show homogeneity in their α-chains, although there are exceptions, as is the case with type I collagen, a heterotrimer composed of two α-1 chains and one α-2 chain (Purslow, 2017). The α-chains that compose collagen are identified by their own components, as well as the collagen in which they are found, and are encoded by different genes. For example,

the type 1 α-chain that is found in collagen III can be written as α-1 (III), while the α-chain 1 that is coded by a different gene and appears in type I collagen would be referred to as α-1 (I) (Bruce and Aalhus, 2017).

The triple helix of collagen is unstable at 37 ºC, which is just below the average body temperature of common livestock, with collagen monomers degrading within a few days of being exposed to body temperature (Leikina et al. 2002). Collagen structures exist throughout the body however, and resist unravelling at body temperature because collagen monomers are incorporated into collagen structures by the action of lysyl oxidase, which forms divalent crosslinks between collagen monomers at specific lysines (Purslow 2017). In 2006, Sun and colleagues used second-harmonic generation microscopy to view the degradation of Type I collagen at varying temperatures. They observed that fibril density was maintained despite evidence of thermal denaturation, showing that while the fibrils may be readily broken down by heat, the bonds between collagen fibrils when stacked in their quarter staggered arrangement within the fibril bundle are more stable at higher temperatures, allowing collagen to maintain its structure in the body. Because some structure was still observed at 54 ºC and the experiment was conducted over 180 minutes, these findings have implications for the degradation of collagen during cooking, especially for quick-cook cuts of meat, where a greater amount of collagen fibril bonding may limit the degradation of collagen and increase the background toughness of meat.

1.9.2 Primary structure of collagen

Glycine appears in the helical portion of α-chains of collagen as the third amino acid in a repeating chain and thus comprises one third of the total collagen content, while the other two amino acids are often proline and hydroxyproline (Pearson and Young, 1989). Other amino acids found in small quantities in collagen are methionine, cysteine, and tyrosine (Purslow, 2017).

Alanine is also found in large amounts, and lysine and hydroxylysine are present as well and play a crucial role in collagen crosslinking (Pearson and Young, 1989).

1.9.3 Collagen crosslinking

Collagen stabilizes its structure through the formation of divalent or trivalent crosslinks (Eyre and Wu, 2005). Collagen synthesis begins in the fibroblast, where the triple helix is formed before entering the extracellular space, and this is where enzymes cleave the end of the procollagen molecule to prepare for secretion from the fibroblast and crosslinking (Wu et al. 2020). The formation of intermolecular crosslinks in collagen is a process that begins with the activity of the enzyme lysyl hydroxylase. Lysyl hydroxylase creates binding sites on lysine where the collagen molecules can attach to one another, either as lysine-hydroxylysine or hydroxylysine-hydroxylysine links, through the hydroxylation of the lysine residues (Kivirikko and Pihlajaniemi, 1998). Lysyl hydroxylase is essential to embryonic development and maintaining the structure of the muscle tissue. Gene knockout mice that did not produce one of the isoforms of lysyl hydroxylase, specifically lysyl hydroxylase 3, grew normally in utero up to day 8.5, then growth was slowed until death on day 9.5 (Rautavuoma et al. 2004). Embryos also showed fragmented basement membranes, which indicates the essential role collagen plays in stabilizing the basement membrane. These results show that the regular function of lysyl hydroxylase which enables collagen proteins to adhere and form an organized structure is crucial for embryonic development and maintaining the integrity of muscle structure, and that a breakdown of this process is lethal.

Lysyl oxidase then catalyzes a reaction on telopeptide lysine or hydroxylysine (residues on the end of the collagen molecule) to add an aldehyde group to the residues and form allysine and hydroxyallysine respectively (Eyre and Wu, 2005). Lysyl oxidase has two cofactors, copper

and ascorbic acid, and the absence of either of these cofactors results in a loss of the stability of collagen (Purslow, 2017). These aldehyde groups then spontaneously bind to helical lysine and hydroxylysine residues within the collagen structure and form divalent crosslinks, which can then go on to spontaneously bind to other helical lysine and hydroxylysine residues and form trivalent crosslinks.

The two major mature collagen crosslinks that have been identified in beef muscle are Ehrlich chromogen (Pyrrole) and pyridinoline, and these mature trivalent crosslinks have been associated with increased beef toughness in older animals (Roy et al. 2015). The current understanding regarding the formation of mature collagen crosslinks is that these occur more often in older animals because there is more time for these trivalent crosslinks to spontaneously form, whereas younger animals have a greater abundance of newly synthesized immature divalent collagen crosslinks (McCormick, 1994). There is evidence that Ehrlich chromogen content decreases with animal age while pyridinoline increases, and both crosslinks are positively correlated with lower collagen solubility and increased toughness in cooked beef in young animals, while pyridinoline continues to be associated with lower collagen solubility in mature beef (Roy et al. 2020).

1.10 MMPs and TIMPs

1.10.1 MMPs

Like myofibrillar proteins collagen can be degraded, and this is performed by matrix metalloproteinases (MMPs), which are zinc-dependant collagenases that are very specific in terms of the types of collagens that they degrade (Nagase and Fields, 1996). Currently there are 25 members of the MMP family that have been identified, and collagen types I-III are cleaved by MMP-1, 2, 8, 13, 14, 18, and 22 (Lauer-Fields et al. 2002).

MMPs cleave collagen types I-III into one-quarter and three-quarter fragments, and the cleavage site has a low content of charged residues and a tighter triple helical structure compared to the loose helix immediately following, which also has a higher content of imino acids (Fields, 1991). The activity of MMPs is highly dependent on specific sequences of collagen. The substitution of isoleucine with proline, for example, has been shown to completely stop the activity of MMP-1, and therefore collagen with this substitution in the cleavage site will not be degraded by MMP-1 (Hasty et al. 1993). In fibrosis research, collagen with hydroxyallysine bonds is less easily degraded by MMP-1, and a higher prevalence of these bonds may influence the irreversibility of fibrosis (Slot-Verhoeven et al. 2005). A higher content of hydroxyallysine bonds in type I collagen in skeletal muscle therefore may increase the background toughness of meat due to the reduced ability of MMP-1 to degrade this collagen.

MMPs are activated via a proteolytic cascade. MMP is produced in its inactive form, where cysteine is bound to zinc, and activation occurs when the bridge between catalytic zinc and cysteine is broken (Brinckerhoff and Matrisian, 2002). Activation is dependent on stromelysin however, and this occurs through the following pathway: keratinocytes produce urokinase, which converts plasminogen to plasmin. Plasmin converts pro-stromelysin to stromelysin, and converts procollagenase to a partially activated collagenase. The partially activated collagenase is then fully activated by stromelysin (He et al. 1989).

MMPs are located in and degrade the extracellular matrix in the living organism, and in doing so they play a role in tissue remodelling. They have been found to remodel tissue during embryonic development, assist in wound healing, and participate in tooth development (Snoek-van Beurden and Von den Hoff, 2018). The breakdown of connective tissue is also needed for muscle development, as collagen networks must be adapted to allow for muscle fiber

hypertrophy. Collagenases such as MMPs thus play an active role in muscle growth (Christensen and Purslow, 2016). MMP activity is normally balanced with the activity of their inhibitors, but when MMPs degrade tissue beyond what is necessary, various pathologies can develop. For example, elevated levels of MMP-1, 2 and 3 have been found in non-healing ulcers (Beidler et al. 2008).

MMPs continue to be active post-mortem, and degrade collagen while meat is refrigerated for post-mortem storage and ageing and may contribute to increased levels of tenderness (Vieseth-Kent et al. 2017). MMP activity appears to be highest in the later stages of beef aging, with collagen degradation being pronounced between days 10 and 14 of post-mortem ageing (Nishimura et al. 1995). Studies of post-mortem ageing of fish muscle found that MMP-2 plays a major role in tenderization at 30 ºC and 4 ºC, and that the addition of the metalloproteinase inhibitor EDTA significantly decreased the softening of muscle in carp (Xu et al. 2015).

However, increasing the activity of MMPs in the living organism does not affect the entire organism equally. The phenotypic expression of MMP activity both in vivo and post-mortem varies between muscles within the individual organism, further degrading collagen in some muscles. The in vitro activity of MMP-2 was studied by Archile-Contreras et al. (2010) who found that MMP-2 activity was highest in the *semitendinosus* muscle when compared to the *sternomandibular* and the *longissimus* muscle. While this study did not evaluate the tenderness of the muscles that were studied, *longissimus* has been found to be more tender than *semitendinosus* (Wheeler et al. 2001), which is unexpected if the activity of MMP-2 is the only factor influencing meat tenderness. Thus, tenderness is multifactorial, and the activity of one

enzyme or a group of enzymes cannot be the only factor being considered when predicting the quality of beef.

1.10.2 TIMPs

MMPs are blocked by tissue inhibitor of metalloproteinases (TIMPs) and this is achieved through binding to the activated catalytic zinc on the MMPs (Brickenhoff and Matrisian, 2002). The function of TIMPs is not as simple as blocking the activity of MMPs, however. TIMP-2 has been shown to activate MMP-2 indirectly, by interacting with other molecules that then cleave the pro-domain of MMP-2 and activate the enzyme (Goldberg et al. 1989). TIMP-2 acts as both an activator and an inhibitor of MMP-2 depending on the concentration of TIMP-2, where it will act as an activator of the collagenase when there is an absence of it and thereby regulates its own activity (Butler et al. 1998).

1.11 Genetic analysis

The advent of new technologies in genotyping and genetic testing has allowed scientists to predict certain phenotypic traits in production animals, and while in most cases no single gene is responsible for multifactorial beef quality traits such as overall colour or tenderness, which are traits that are sometimes subjective when evaluated by a sensory panel, selection for certain genes that affect the traits of interest may still be used to produce high quality meat. By analyzing a genome wide association study (GWAS), Chen et al. (2015) were able to find that fatty acid content of beef was influenced by a few major genes, with limited influence by a large number of other genes. This finding could be used to genetically select for desired phenotypes by screening for the presence of those genes which give rise to the desired effect in meat.

The heritability of desired traits must also be taken into account when considering genetic selection for meat quality. Zwambag et al. (2013) found that the heritability of meat tenderness

decreased with aging time, from 0.194 at 7 days post-mortem to 0.048 at 21 days post-mortem. The authors noted that previous estimates of the heritability of meat tenderness have varied widely, and this is most likely due to how easily meat tenderness is affected by pre- and post-slaughter conditions. The low shear force scores and low heritability of tenderness at 21 days post-mortem indicates clearly in this study that post-mortem aging is a valuable tool for improving meat tenderness as it can decrease the effect of animal genetics on meat tenderness. Unfortunately, little study has been done to estimate the heritability of collagen characteristics of beef, though the heritability of total collagen was estimated to be approximately 15% in meagre fish (Vallecillos et al. 2021) with high standard error.

The use of genetic analysis can also lead to finding new correlations between carcass traits and genes, which allows scientists and producers to target different genes to improve the quality of the meat product. Dunner et al. (2013) performed a GWAS on 15 European beef breeds, and in so doing were able to identify single nucleotide polymorphisms (SNPs) that were economically valuable, as they were correlated with phenotypic differences in carcass and meat quality traits such as juiciness, collagen content, and the calpain/calpastatin system. The SNPs in the MMP-1 gene that are associated with improved carcass traits are a novel finding of this study, and MMP-1 activity was found to be linked to calpain and calpastatin activity.

SNPs do not have to occur within a gene influencing a certain process to have an effect on that process, however, as alterations to other proteins both up and downstream through the expression level of a gene can influence phenotypes. Calpain-2 or m-calpain has been linked to MMP-1 activity (Yang et al. 2017), where microRNA-93 and collagen I were downregulated when expressions of MMP-1 and m-calpain were found to be upregulated. MicroRNA-93 was also able to bind to calpain-2 and overexpression of microRNA-93 decreased the expression of

calpain-2. Alterations to the coding region for microRNA-93 may influence collagenase activity and collagen synthesis through the expression of calpain-2.

Individual genes can also have pleiotropic effects, where the expression of a single gene effects multiple mechanisms and pathways. *Transforming growth factor β1 (TGF-β1)* has been linked to the formation of scar tissue in flexor tendon injuries, and the mechanism by which this occurs has been studied by Farhat et al. (2012), who theorized that expression of this gene favors synthesis of the extracellular matrix. It was found that the expression of *TGF-β1* downregulated the expression of the collagenase MMP-16 and upregulated collagen V, collagen XII, and Plasmogen Activator Inhibitor 1, a tissue inhibitor of MMP.

1.12 Production technologies that affect beef toughness

There are many different commercially available growth promoters that are approved for use in the beef industry that aim to increase the average daily gain of beef cattle and improve feed efficiency, which is the ratio between the weight an animal gains and the food it consumes. Studies have shown that growth promoters can have detrimental effects on meat quality, with beef from implanted cattle having higher mean WBSF values than untreated controls (Scheffler et al. 2003).

Steroidal implants vary in potency and tend to be used at different stages of animal growth depending on their potency. In Canada, a low potency implant containing a combination of estrogen and progestin is used in suckling calves that are less than 182 kg, while a moderate potency implant containing more estrogen than the one with lower potency would be given to cattle that have been weaned and weigh more than 182 kg (Smith and Johnson, 2020). An implant that contains a combination of estrogen and trenbolone acetate would be used as a terminal implant, and these tend to be highly potent.

Beta-adrenergic agonists such as zilpaterol hydrochloride and ractopamine have also been shown to affect beef toughness and solubility of collagen and have been studied in conjunction with steroid implantation because the two technologies are often used in tandem in finishing cattle. A study by Kellermeier et al. (2009) aimed to evaluate the effects of the β-agonist zilpaterol hydrochloride (ZH) on beef tenderness when used in the last 30 days prior to slaughter of beef cattle. This experiment looked at the effects of ZH alone, ZH in combination with an estrogen and trenbolone acetate implant, and the steroidal implant alone in comparison to untreated beef. Use of both ZH and the implant together, as well as ZH on its own and the implant on its own significantly increased the WBSF of meat compared to the untreated samples. Collagen content was decreased in samples that were treated with ZH alone, however, the collagen content of samples from cattle treated with both ZH and the steroidal implant did not differ significantly from the untreated control group. In this study, collagen content did not appear to significantly affect meat tenderness as evaluated by WBSF, although the authors suggested that this may be due to the muscle fiber hypertrophy that results from the use of ZH having a dilution effect that decreases the concentration of collagen in the muscle.

One concern with beef production is the environmental impact of the practice, as well as the contributions of cattle rearing to greenhouse gases, so there is an interest in producing beef product more quickly and with more efficient use of resources. A recent study by Capper et al. (2021) aimed to illustrate the benefit of steroids in beef production in order to reduce the environmental impact of the practice, as well as the economic cost of feeding and housing animals prior to slaughter. Animals were categorized into 4 groups: non-implanted controls, and low, medium, and high levels of enhancement. Compared to the non-implanted controls, the feed, water, land, and fossil fuel costs of producing the same amount of meat was significantly

lower in implanted cattle, with resource cost decreasing as enhancement level increased. The return on investment for producers was also higher in implanted cattle. It is clear then that the use of steroids in cattle can address public concern regarding the environmental cost of producing meat, but care must be taken to avoid the negative effects of steroids on meat quality.

1.13 Conclusions, hypotheses, and objectives

As the industry continues to improve the procedures involved in the care of beef cattle, and variation between production systems is limited to produce the highest quality of beef possible, the genetic makeup of the animals in production systems must be considered to make further progress. Consumers are willing to pay a premium for beef products that have a guarantee for tenderness (Miller et al. 2001), and so there would be a benefit to producers to raise animals that have the genetic traits that produce a tender phenotype.

Collagen has been shown to contribute to increased beef toughness, especially when total collagen content is high and the solubility of the collagen is low, as is the case in older animals (Roy et al. 2020) and when there is a presence of mature trivalent collagen crosslinks (Roy et al. 2015). As MMPs function as collagenases and can cleave collagen molecules despite the presence of mature crosslinks (Giannobile, 1999), it would be economically valuable to select for genotypes that have improved MMPs activity and have been shown to produce cuts of meat that have lower toughness scores due to a decrease in collagen residues.

As the research by Dunner and colleagues has shown (2013), SNPs have been identified within the genes affecting MMP, lysyl hydroxylase, and total collagen content, but the effect of these SNPs has yet to be evaluated. It is possible that selecting for specific SNPs could result in increased tenderness in beef meat and therefore increase the quality of these cuts. As MMP-1 was also found in this study to be associated with calpain activity, it is possible that selecting for

MMP activity may also improve the activity of the calpain system, which is also involved in the tenderization of meat post-mortem as previously discussed. These SNPs have yet to be evaluated, and research that focuses on genetic influences on collagen characteristics in meat has not been conducted.

Based on this review, the present research will test the following two hypotheses:

1. Post-mortem aging of beef increases collagen solubility, and
2. SNPs exist in genes related to MMPs and collagen synthesis that are related to decreased toughness of cooked beef and increased collagen solubility.

The thesis will focus on the *m. longissimus lumborum* (strip loin) which, although it is a low to moderate collagen content muscle, tends to be tender (Shöenfeldt and Strydom, 2011) and is therefore an expensive cut that is usually cooked quickly. This gives little time for the collagen to be solubilized by heat into gelatin, making collagen content a factor that affects the tenderness of this muscle.

The objectives of this study are therefore to:

1. Characterize the change in collagen solubility before and after post-mortem ageing of beef *longissimus lumborum;*
2. Estimate the heritability of collagen traits and the genetic correlations between those traits to better understand the influence of genetic controls on collagen, and improve the design of selection programs; and
3. Relate collagen solubility before and after post-mortem aging, as well as collagen crosslinking, to cattle genotype to identify SNPs associated with increased collagen solubility that can serve ultimately as gene markers for decreased toughness of beef.

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Chapter 2: Materials and Methods

2.1 Animals and experimental design

A total of 198 *M. longissimus thoracis et lumborum* (LT) muscle samples, one from each of the carcasses of 198 crossbred beef steers were used in this experiment. The steers were from two locations, with 49 from the University of Guelph beef research facility at Elora, Ontario, Canada, and 149 from the Agriculture and Agri-Food Canada facility at Lacombe, Alberta, Canada. The 49 steers located at the University of Guelph were fed a growing diet for 4 months followed by backgrounding on pasture, and then were given a finishing diet prior to slaughter (Table 2.1). The nutrient breakdown of the diet is described in Table 2.2. Steers were approximately 2 years of age at slaughter and did not receive steroids. Cattle from Guelph were slaughtered at the Cargill Guelph facility in accordance with federal regulations (Canadian Justice Laws Website, 2022). The Guelph carcasses were subjected to electrical stimulation (low voltage in the hide down puller followed by a high voltage prior to entering the hot box, with exact voltages proprietary) and a Tendercut^{TM} procedure as described in Sørheim and Hildrum (2002). Briefly, two cuts were made to the pre-rigor carcass just after slaughter, with one cut between the 12th and 13th thoracic vertebrae severing the *multifidus dorsi,* and the other cut was made between the sirloin and round between the 4th and 5th sacral vertebrae, severing the *ischium* of the pelvic bone. The Achilles tendon suspension was maintained, and the two cuts stretched the major loin and round muscles, with the intent being to improve tenderness and reduce the effects of cold shortening as rigor progresses.

Table 2.1: Ingredient composition of diets (dry matter basis)

Ingredient	Growing diet DM basis (%)	Finishing diet DM basis (%)
Alfalfa silage	73.1	15.3
Corn silage	25.7	----
FFM beef feedlot premix	1.0	1.1
White salt	0.2	0.3
High moisture corn	-----	76.5
Soybean meal	-----	6.8

Note: FFM (Floradale Feed Mills Ltd., Floradale, ON) beef feedlot premix contained: 24% calcium, 3.0% phosphorus, 3.55% sodium, 1.95% magnesium, 4.0% potassium, 2.3% sulphur, 27 mg kg^{-1} cobalt, 74 mg kg^{-1} iodine, 1765 mg kg^{-1} iron, 750 mg kg^{-1} copper, 280 mg kg^{-1} fluorine, 3000 mg kg^{-1} manganese, 3000 mg kg^{-1} zinc, 460 KIU kg^{-1} vitamin A, 114 KIU kg^{-1} vitamin D, 2075 IU kg^{-1} vitamin E, and 33 mg kg^{-1} rumensin.

Table 2.2: Chemical composition of diets (dry matter basis)

Ingredient	Growing diet DM basis (%)	Finishing Diet DM basis (%)	Pasture DM basis (%)
Dry matter (%)	33.7	62.5	22.9
Metabolizable energy (Mcal kg^{-1})	2.31	2.96	2.33
NE maintenance (Mcal kg^{-1})[a]	1.44	2.01	1.46
NE gain (Mcal kg^{-1})[a]	0.85	1.35	0.87
Crude protein (%)	16.2	13.2	16.4
Starch (%)	8.3	48.9	
Neutral detergent fibre (%)	39.8	15.1	48.0
Acid detergent fibre (%)	32.6	9.6	31.9
RUP (% of crude protein)[1]	21.4	25.3	
NDF-CP (%)[2]	2.25	0.67	
ADF-CP (%)[3]	1.89	0.8	
Calcium (%)	1.51	0.56	
Phosphorus (%)	0.37	0.40	
Potassium (%)	2.15	0.99	
Magnesium (%)	0.34	0.21	
Copper (ug g^{-1})	16.7	1.18	
Zinc (ug g^{-1})	61.5	60.2	
Iron (ug g^{-1})	515.1	133.1	

[1]RUP: Rumen undegraded protein
[2]NDF-CP: Neutral detergent fiber – crude protein
[3]ADF-CP: Acide detergent fiber – crude protein

The 149 beef steers raised at the Agriculture and Agri-Food Canada (AAFC) facility in Lacombe were “soft” weaned using a see-through barrier fence in September and then fed barley silage until being divided into calf and yearling fed groups. The steers in the calf fed group were

switched to the feedlot ration in early spring while the yearling fed steers were sent to pasture. The yearling fed steers grazed until September and then were brought in and put onto the feedlot ration. All steers were fed a feedlot ration once they adapted to full feed (3-4 weeks), which consisted of 78% steam rolled grain (Masterfeeds, Ontario, Canada) and 22% barley silage. The steers were fed in the feedlot for 100+ days. Of the 149 steers in this study, 64 were yearlings (662 days of age ± 44) and 85 were calves (496 days of age ± 35) at slaughter, all of which were involved in a steroidal implant treatment study. Cattle were slaughtered at the Agriculture and Agri-Food Canada Meat Laboratory in Lacombe federally inspected slaughter facility using the captive bolt method in accordance with federal regulations (Canadian Justice Laws Website, 2022).

2.2 Muscle collection

All LT muscles were harvested at 48 hours (2 days) postmortem. At 6 days postmortem, a steak was collected from the LT muscle between the 6^{th} and 7^{th} ribs and between the 12^{th} and 13^{th} ribs by staff from the University of Guelph and AAFC, respectively. The Guelph samples were between 8-9 cm thick sections and the Lacombe samples were 2.5 cm thick steaks. The samples were vacuum packed and frozen at 7 days postmortem (dpm) and transported to the laboratory at the University of Alberta, where they remained frozen until thawed in preparation for aging for a further 14 days. Samples were removed from the freezer in random order for thawing, aging and further analysis.

2.3 Sample group study allocation

Because the cattle populations underwent different treatments, meat quality characteristics were studied by population, with steers from the Guelph study constituting Study 1 and those from the AAFC study constituting Study 2. Study 1 was conducted as an

observational study as all steers received similar treatment and no steroid implants, so data from steers in study 1 were interrogated for the effects of yield grade and quality grade on meat quality. Canadian quality grade is determined using animal age, sex, fat colour, texture, and cover, as well as meat colour and marbling (Beef Cattle Research Council, 2022). The samples in study 1 had Canadian quality grades of Prime (marbling is slightly abundant), AAA (small amount of marbling), and AA (slight amount of marbling). Yield grade is applied to Prime and any A grade carcasses, with estimated retail yield decreasing as the grade increases from 1 to 5 (Beef Cattle Research Council, 2022). No animals in study 1 had a yield grade of 1. Yield grade 2 puts the carcass yield that is saleable at retail at 52.34-50%, grade 3 is 50-47.7%, 4 is 47.7-45.4%, and carcasses with a yield grade of 5 have an estimated saleable yield of less than 45.4%.

Study 2 was conducted with a specific experimental design with planned comparisons, where the application of different steroids at different production ages was investigated. To control for a possible year effect, as study 2 was comprised of two cattle populations born in different years, each year was evaluated individually, with the steers born in 2017 comprising study 2A, and the population born in 2018 comprising study 2B. Study 2A involved six treatments, where steers were ranked by weight and then randomly assigned to one of the six treatments. One treatment group (calves, n = 8) received a terminal implant composed of 120 mg trenbolone acetate and 24 mg estradiol (Component TE-S, Elanco Animal Health, Guelph, Ontario) at 100-129 days prior to slaughter (TA). A second treatment group (yearlings, n = 16) received one Component E-S (Elanco Animal Health, Guelph, Ontario) implant, a backgrounding implant composed of 200 mg progesterone and 20 mg estradiol benzoate at approximately 13 months old (394 days old ± 15.9) (PE1), while a third treatment group received two Component E-S implants, the second of which was administered 89 days after the first

(yearlings, n = 16) (PE2). A fourth treatment group (yearlings, n = 16) received the initial implant at approximately 13 months of age, and the terminal implant 100-129 days before slaughter (PE1T). A fifth treatment group received two Component E-S implants 89 days apart and a terminal Component TE-S implant pre-slaughter (yearlings, n = 16) (PE2T). The sixth group was a control group that did not receive steroidal growth hormones (calves, n = 47). In Study 2B thirty (calves, 30) steers received the terminal implant, and there were 31 control (calves, 31) steers. The implant treatments of study 2A and 2B are described in Table 2.3.

Table 2.3: Treatments in the Lacombe steroidal implant experiments

	Component E-S (13 months)	Component E-S (16 months)	Component TE-S (100-129 days pre-slaughter)
Study 2A			
Control 2017 (N = 16)	None	None	None
TA (N = 8)	None	None	Implanted
PE1 (N = 16)	Implanted	None	None
PE2 (N = 16)	Implanted	Implanted	None
PE1T (N = 16)	Implanted	None	Implanted
PE2T (N = 16)	Implanted	Implanted	Implanted
Study 2B			
Control 2018 (N = 31)	None	None	None
Treated 2018 (N = 30)	None	None	Implanted

Additionally, collagen data and genotypes from 137 *gluteus medius* samples from a previous study (Lei, 2019) were used in a genome-wide association study in combination with the data derived from the AAFC samples of the current study. A description of the animals, specifics of their production and slaughter, and the analyses associated with characterization of their muscles were described by Lei (2019).

2.4 Sample allocation

Each LT muscle sample was thawed overnight at 4 ºC and divided into 3 parts for further testing (Figure 1). Due to differences in sample shape and mass and to accommodate planned

treatments, LT from Guelph were divided from caudal to cranial end of the muscle section (Figure 2.1a), while LT from Lacombe were divided from the dorsal to the ventral sides of the single steak provided (Figure 2.1b). The first two parts of each sample regardless of location were used for soluble and insoluble collagen quantification, where the first cut was processed at 7 dpm and the second cut was packaged under vacuum in polypropylene bags and aged for 14 days at 4 ºC for a total of 21 dpm. The third portion was retained, considered aged for 7 dpm, and immediately used for isolation of intramuscular connective tissue.

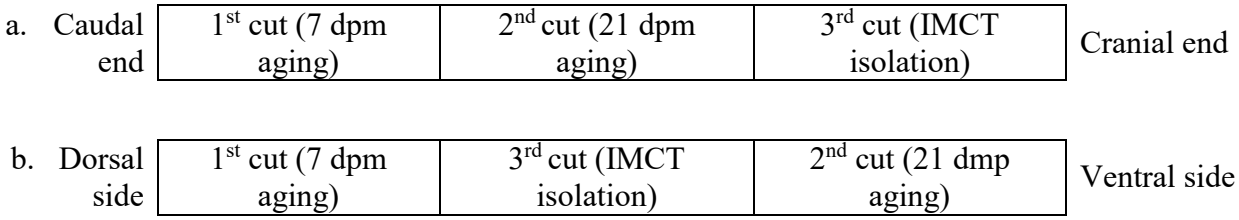

Figure 2.1: a: Location of LT muscle subsamples used for soluble collagen and collagen cross-links determination. Guelph samples were collected from between the 6th and 7th ribs. b: Location of LT muscle subsamples used for soluble collagen and collagen cross-links determination. Lacombe samples were collected from between the 12th and 13th ribs.

2.5 Collagen solubility

2.5.1 Preparation of freeze-dried meat for soluble collagen

The first two sections of each muscle sample were designated for use in assessing collagen solubility at 7- and 21-days ageing. Freezing of the samples at 7 days was deemed to have halted ageing, and thawing was deemed to have initiated it again; therefore, ageing a further 14 days after thawing was considered to constitute a total of 21 days post-mortem ageing. For each section, muscle sub-samples were trimmed of external fat and epimysium, cut into 1 cm cubes and placed in an aluminum tray covered with aluminum foil of known weight that was vented with perforations to allow for moisture to escape. The samples were frozen at -20 ºC prior

to lyophilization, then dried for 7 days. Samples were weighed in the trays with the foil lids after drying to determine moisture loss, with the weight of the tray and lid subtracted from the total weight to determine the weight of the sample. The freeze-dried samples were ground to a fine powder using a stainless-steel domestic blender, into which a piece of dry ice was added to avoid the accumulation of heat during blending. Samples were then stored at -20 ºC until further analysis.

2.5.2 Extraction of soluble and insoluble collagen from freeze-dried meat

The Hill (1966) method was used to extract soluble collagen with some modifications. About 1 g (1.0013 ± 0.0008 g) of lyophilized meat was homogenized with 20 mL of ¼ strength Ringer's solution and incubated in a water bath at 77 ºC for 60 minutes, with additional time allowed for the water bath to reach 77 ºC. The solution was then centrifuged (Avanti J-E Centrifuge, Beckman Coulter, Mississauga, ON) at 3500 *g* for 10 minutes, after which the supernatant containing the soluble collagen fraction was collected for determination of soluble collagen content. The tubes containing the residue were then inverted at a 45-degree angle and left to dry for 30 minutes, after which their weight was recorded, and the residue was collected for use in determining insoluble collagen content. Residue weight was determined by subtracting the weight of the dry centrifuge tubes from the recorded weight of the tube with residue. Soluble collagen was determined as an average of two duplicates for all samples while insoluble collagen was quantified as an average of two duplicates for the Guelph muscle samples only. Bruce et al. (2022) indicated that total collagen calculated from hydrolyzed IMCT as part of the quantification of pyridinoline was more representative of total collagen than quantification of hydroxyproline from the sum of soluble and insoluble collagen.

2.5.3 Hydrolysis of soluble and insoluble collagen

For acid hydrolysis, 1 mL of soluble collagen supernatant was combined with 1 mL 12 M HCl and 3 mL 6 M HCl in a 20 mL glass screw-top test tube to bring the solution to 6 M. The contents were mixed by vortex. For insoluble collagen, about 0.30 g of residue was combined with 5 mL of 6 M HCl (Study A samples only) in a similar test tube and mixed by vortex.

The tubes were then purged with nitrogen gas and capped with Teflon caps. Samples were then hydrolyzed for 20 hours at 110 ºC. The tubes were then chilled in ice water for 15 minutes to halt hydrolysis. The hydrolysates were filtered with No. 4 Whatman filter paper and evaporated to dryness using a rotary evaporator in a water bath set at 50ºC and under 690 mmHg vacuum (Heidolph Collegiate rotary evaporator equipped with a DistiVac Ultra auto-purge vacuum system, Brinkmann, Mississauga, ON). The samples were then reconstituted with 2 mL of deionized (DI) water and neutralized with 2.5 M NaOH, with further adjustments made using 0.5 M NaOH and 0.1 M HCl until the reconstituted samples reached a pH of 7 as determined using litmus strips. The neutralized samples were then once again evaporated to dryness and reconstituted to a final volume of 5 mL with DI water. Samples were hydrolyzed in duplicate.

2.5.4 Hydroxyproline assay for collagen quantification

Collagen quantification was performed by quantifying the concentration of hydroxyproline in the hydrolysates. Hydroxyproline was determined using the hydroxyproline assay described by Neuman and Logan (1950). One mL of the reconstituted collagen hydrolysate (soluble or insoluble) was placed in a glass test tube, to which the following solutions were added in order: 1 mL of copper (II) sulfate (0.01 M), 1 ml of 2.50 N sodium hydroxide, and 1 mL 6 % hydrogen peroxide. The composite solution was then left to stand for 5 minutes at room temperature with occasional swirling and was then transferred to an 80 ºC water bath, where it

was shaken vigorously until hydrogen peroxide was completely removed as indicated by a colour change which occurred after about 2 minutes. The tubes were then placed in an ice bath to cool. Once cooled, 4 mL of 3.0 N sulfuric acid and 2 mL of 5 % (w/v) p-dimethylaminobenzaldehyde in isopropanol (A451-4 HPLC grade 99.9% assay 2-propanol, Fisher Scientific, Mississauga, Ontario) were added and the tubes were mixed by vortex. The tubes were then placed in a 70 ºC water bath for 16 minutes, after which they were immediately removed and placed into a tap water bath for 5 minutes and allowed to cool to room temperature. A blank was prepared similarly using DI water in place of sample, and hydroxyproline standard from a hydroxyproline standard stock solution (400 ppm, L-trans-4-hydroxyproline) was prepared with the following concentrations: 2.5, 5, 10, 20, and 40 µg/1 mL. Absorbance was measured against the blank at 550 nm. A standard curve was generated by regression of the absorbances of the standards onto their known concentrations of hydroxyproline (Excel™, Microsoft Corporation) and the equation of the curve was used to quantify the hydroxyproline content in the muscle samples based on their absorbances. All samples and standards were prepared in duplicate. The hydroxyproline content determined by this assay was converted into collagen concentration using a factor of 7.14 with the assumption that hydroxyproline content of intramuscular collagen is 13-14% based on the findings of Stanton and Light (1987).

2.6 Isolation of IMCT

At 7 dpm, the third cut was trimmed of epimysium and cut into cubes for IMCT isolation. External fat and epimysium trimmings were conserved for animal genotyping.

Intramuscular connective tissue was isolated according to the methods described by Roy et al. (2015) with some modifications. Cubed muscle (approximately 200-300g, with the amount varying depending on size of the sample) was weighed and the weight recorded. It was then

homogenized in a laboratory blender (Waring, New Jersey) with 5 volumes of deionized (DI) water chilled at 4 ºC. Blending was performed on low speed for 10 seconds, then on high speed for 10 seconds. The resultant slurry was passed through a metal sieve (1 mm^2 pore size), the material left on the sieve was collected as IMCT, and lipid and non-collagenous protein discarded. This tissue was blended again with DI water and passed through the sieve as before to further purify, again rinsed with DI water at 4 ºC, then patted dry with No. 4 Whatman filter paper (Fisher Scientific, Mississauga, Ontario). Any blood vessels that remained in the tissue were removed manually. The wet IMCT was weighed and frozen at -20 ºC until defatted and dried. Prior to defatting, the wet IMCT was thawed at about 4 ºC and then treated with a solution of chloroform and methanol (2:1 v/v) to remove fat. Wet IMCT was soaked in the chloroform-methanol solution at a ratio of 1:10 (w/v) for 1 hour with constant stirring using a magnetic stirrer. This process was repeated twice where the solution containing lipids was discarded and fresh chloroform-methanol solution was added for each repetition, with the final treatment lasting for 1.5 hours. Treated IMCT was dried overnight in a fume hood at room temperature, after which the dry IMCT weight was recorded, and the dry IMCT was frozen at -20 ºC until analysis.

2.7 Ehrlich Chromogen collagen crosslink quantification

The concentration of Ehrlich Chromogen (EC) crosslink was determined according to the method described by Roy et al. (2015). Approximately 100 mg of defatted and dried IMCT were suspended in a 50 nM Tris-HCL, 1 mM KCl solution at a concentration of 20 mg/mL. The samples were allowed to hydrate in the solution overnight at 4 ºC. The suspensions were then heated in a 65 ºC water bath for 1 hour to denature the sample, and mixed by vortex at 0, 15, 30, and 45 minutes. They were cooled to 37 ºC in a tap water bath, after which trypsin (TPCK

treated from Sigma Aldrich) solution was added (0.5 mg trypsin/ 1 mL sample) and the suspensions were incubated at 37 ºC for 4 hours, and vortexed every 15 minutes. They were then immediately heated in a 65 ºC water bath for 20 minutes to render the trypsin inactive. The samples were cooled to room temperature (25 ºC), centrifuged (Avanti J-E Centrifuge, Beckman Coulter, Mississauga, ON) at 28000 *g* for 10 minutes, and filtered through a 0.45 µm nylon filter. One mL of filtrate was added to 200 µL of 5 % (w/v) p-dimethyaminobenzyldehyde in 4 N perchloric acid containing 0.01 % mercuric chloride and centrifuged for 2 minutes at 14000 *g* to remove any turbidity. The absorbance of the samples was measured at 572 nm and 640 nm, where the 640 nm reading was considered the baseline and subtracted to determine the actual reading at 572 nm (Ngapo, 2002). The samples were measured against a blank that contained 60% perchloric acid and deionized water (acid: water = 44:56 v/v) with 0.01% mercuric chloride.

Ehrlich chromogen crosslink concentration was expressed as mol EC/mol collagen using a molar extinction coefficient of 25000 (Kemp and Scott, 1988). The molar concentration of EC crosslink in tryptic digesta was calculated according to Beer-Lambert law $A = \varepsilon Cl$, where A is the absorbance, ε is the extinction coefficient, C is the concentration, and l is the length of the path of light through the cuvette in cm. As the cuvette is 1 cm, the molar concentration was simply expressed as A/ε. The remaining tryptic digesta was conserved and held at -20 ºC for use in the hydroxyproline assay for the quantification of total collagen in tryptic digesta. The assay was performed in duplicate.

2.8 Pyridinoline collagen crosslink quantification

For the quantification of pyridinoline, about 0.2 g of IMCT was hydrolyzed in 6 mL of 6 M HCl in a 110 ºC dry bath for 20 hours in a Teflon capped glass tube purged with nitrogen gas. After hydrolysis the samples were cooled for 15 minutes in an ice water bath and then filtered

using Whatman No. 4 filter paper (Fisher Scientific, Mississauga, Ontario), after tubes were rinsed with 2 mL DI water. From the total 8 mL filtrate, 1 mL of hydrolysate was taken to be used for the hydroxyproline assay to determine total collagen. Total collagen was used to express mole pyridinoline per mole collagen. Pyridinoline content was measured using the methods described by Roy et al. (2015). Hydrolysates were minimally exposed to light to avoid degradation of the pyridinoline (Sakura et al. 1982). The hydrolysate was evaporated to dryness using a rotary evaporator at 690 mmHg with a 50ºC water bath (Heidolph Collegiate rotary evaporator equipped with DistiVac Ultra auto-purge vacuum system, Brinkmann, Mississauga, ON), and reconstituted with 1.5 mL 10 % acetic acid, then purified by size exclusion chromatography using gravity in Bio-gel P2 (Bio-Rad, Canada) columns (20 mL bed volume, 1.5 x 12 cm column). The hydrolysates were eluted using 10 % acetic acid, and fractions (6mL) were monitored for pyridinoline using fluorescence with an excitation (λex) of 295 nm and an emission (λem) of 395 nm (Lumina, Thermofisher, USA). Fractions that indicated fluorescence were pooled, evaporated using a rotary evaporator at 50ºC and 690 mmHg, and reconstituted with 0.1 M HCl. Pyridinoline was then further purified using cation exchange chromatography (cellulose phosphate, Sigma Aldrich, 20 mL bed volume, 1.5 x 12 cm column) where columns were first washed with 0.1 M HCl to eliminate contaminants. Then the column was eluted with 1.0 M HCl and pyridinoline containing fractions were detected based on their fluorescence (λex = 295 nm, λem = 395 nm). Pyridinoline containing fractions were then pooled, evaporated to dryness using a rotary evaporator (Heidolph Collegiate rotary evaporator equipped with a DistiVac Ultra auto-purge vacuum system, Brinkmann, Mississauga, ON), and reconstituted with 1.5 mL of 1 % aqueous heptafluorobutyric acid (Sigma-Aldrich Canada, Ltd), where

pyridinoline crosslink content was then quantified using reverse-phase high performance liquid chromatography (Roy et al. 2015).

Reverse-phase HPLC separation was performed at room temperature (25 ºC) in a C_{18} column (Eclipse XDB-C18, 5µm, 4.6 x 150mm) that had been fitted with a guard column (Supelcosil-C18, 20-40 µm, 4.6 x 50 mm). Two solvents were used in the analysis: solvent A, a solution of 0.13 % HFBA in 22 % methanol, and solvent B, 0.1 % HFBA in 75 % acetonitrile. The column was equilibrated with solvent A at a flow rate of 1 mL/min. For the elution of pyridinoline, the column was washed for 30 minutes with solvent A, then solvent B was put through for 10 minutes. The natural fluorescence of pyridinoline was monitored at λex = 296 nm and λem = 396 nm and integrated with chromatography software. The pyridinoline peak was compared to that of a standard curve created using known amounts of pyridinoline (standard pyridinoline, Quidel Corporation, San Diego, CA, USA), and molar pyridinoline was calculated in perimysium as 429 g pyridinoline/mol. All quantifications were performed in duplicate.

2.9 Determination of total collagen

Total collagen was determined in the Study A samples by calculating the sum of soluble and insoluble collagen. Total collagen in IMCT was determined for all samples using the hydroxyproline assay as previously described for both tryptic digesta and the pyridinoline assay. For the tryptic digesta, 1 mL of filtrate was hydrolyzed with 1 mL of 12 M HCl and 4 mL of 6 M HCl, evaporated and reconstituted as previously described. For hydrolyzed IMCT, the 1 mL of filtrate set aside in the pyridinoline assay was evaporated and reconstituted as previously described. For both tryptic digesta and pyridinoline, 50 µL of reconstituted sample was diluted with 950 µL of deionized water to make up 1 mL total, and the hydroxyproline assay was performed as previously described.

2.10 Meat quality

Meat quality data from the steers studied were obtained from researchers at Guelph and Lacombe, respectively. From Guelph, intramuscular pH and bloomed surface colour data at 120 to 144 hours post-mortem were provided, along with marbling score and IMF content from steaks that were aged for 7 days before freezing for storage prior to analysis. Marbling score was evaluated by a meat grader trained by the Canadian Beef Grading Agency using a visual scoring method when the carcasses were scored for quality and yield grade. Surface colour data was collected using the CIELab system of the Chroma Meter CR-400 (light source A, observer angle 10°, aperture size 5 mm, Konica Minolta Sensing, Inc., Tokyo, Japan) after the colorimeter was calibrated using white and black tiles, and a colour reading was taken at six different places on the surface of the sample, with the final measure being an average of the six readings. The pH of the samples was determined using a Hanna Meat pH Meter (model HI98163, probe FC2323, Hanna Instruments Inc., Rhode Island, USA), and was an average of three measurements in three different locations. IMF was measured using the ether extraction method as described by Bureš and Bartoň (2018), where samples are dried and pulverized, and IMF was extracted from the samples using petroleum ether (Soxtex Avanti 2055, FOSS Tecator AB, Höganäs, Sweden). Lacombe provided pH and carcass temperature data at 45 minutes and 72 hours post-mortem, as well as bloomed surface colour at 6 days post-mortem. Steaks were allowed to bloom for 20 minutes at room temperature before colour readings were taken. Surface colour was measured using a Minolta CM-700D meter (light source A, observer angle 8°, aperture size 8mm, Konica Minolta Sensing, Inc., Tokyo, Japan), and was an average of 3 measurements. The pH and temperature of the samples were measured once at both time points using a Fisher Scientific Accumet 1002 pH/temperature meter that was equipped with an Orion Ingold electrode, where

the pH meter probe was inserted into small incisions made in the sample and allowed time to stabilize before a reading was recorded. Warner-Bratzler shear force values for each animal were also provided for the Guelph samples. The methods for obtaining these measures are detailed in Fonseca et al. (2022). Briefly, steaks were cooked to an internal temperature of 70 °C after which they were immediately chilled on ice to halt the cooking process. Eight cores of approximately 1.5 cm were removed parallel to the muscle fibers for each steak and sheared using a Warner-Bratzler blade on a TA-TCT Plus Texture Analyzer (speed of 20 cm/minute, Texture Technologies Corp., Scarsdale, NY). Shear force values were determined as an average of the eight cores from each steak. The AAFC facilities collected Warner-Bratzler shear force data for the samples using a similar method as described, although cores were 1.9 cm and the TA-XT Plus Texture Analyzer (speed of 20 cm/minute, Texture Technologies Corp., Scarsdale, NY) was used.

2.11 Genetic microarray and quality control

Genotyping was performed from tissue-derived DNA using the Illumina GGP Bovine 100K beadchip, which features a total of 101 220 single nucleotide polymorphisms (SNPs). The ARS 2.1 bovine assembly was used to specify SNP positions for use in genomic analysis. SNPs were excluded from the dataset if the missing rate exceeded 10%, the minor allele frequency was less than 5%, or if there was extreme deviation from the Hardy-Weinberg equilibrium ($P < 10\text{-}6$). Animals were excluded from the dataset if they failed to meet a 90% call rate. After quality control there were 149 animals and 95 165 SNPs available for genomic analysis.

2.12 Combination analysis

Genomic analysis was performed on a combined dataset composed of the 149 *longissimus thoracis* samples from animals sourced out of Lacombe and analyzed with the GGP

Bovine 100K beadchip, and 137 samples from a previous study (Lei, 2019). The 137 samples of the previous study were gluteus medius samples from Kinsella steers born between 2013 and 2014 and managed at the Roy Berg Kinsella Ranch, University of Alberta, Canada. They were analyzed using the Illumina Bovine SNP50_v2 Beadchip, which features 54 609 individual SNPs. After quality control 43 450 SNPs were left for use in the genome-wide association analysis. There was an overlap of 31 619 SNPs between the two beadchips, and 29 698 SNPs remained after quality control.

2.13 Non-genomic statistical analysis

Meat quality and collagen data were analyzed initially for the effect of production treatment. Data analyses were performed using the general linear model (GLM) procedure in R studio software (version 4.1.1). For the cattle from Guelph, meat quality and collagen characteristics data were treated as observational and the data were putatively categorized into quality and yield grades, which were considered fixed effects in two separate analyses respectively. Least squares mean differences were used to determine differences in means for yield and quality grade, with differences significant at $P < 0.05$ using Tukey's Honestly Significant Difference (HSD) in the agricolae package (version 1.3-5). Non-significant interactions at $P > 0.05$ within a model that was significant were not considered. Because the data were observational, confidence intervals (95%) were calculated to determine differences between means using an Effect Size Calculator (http://www.campbellcollaboration.org/escalc/hmtl/EffectSize/Calculator-SMD1.php) as is recommended for observational data (Nakagawa and Cuthill, 2007). Within this analysis, quality and yield grade means were compared to Canada AA and Yield Grade 5. Additionally, Pearson

correlation coefficient analysis was performed to identify linear relationships between collagen and meat quality characteristics using the Hmisc package (version 4.6-0).

The genomic breed composition of the animals was then estimated using a database of 20 670 SNPs from a reference population of 4719 cattle, which is composed of the following breeds: Angus-Black, Angus-Red, Brown-Swiss, Charolais, Galloway, Gelbvieh, Hereford, Holstein, Jersey, Limousin, Maine-Anjou, Salers, Shorthorn, and Simmental. Animals in the test population had their genotype panels reduced to match the SNPs of the reference population, and the breed prediction was performed using Admixture software (https://dalexander.github.io/admixture/) (Alexander et al. 2009). The estimated genetic breed composition of the cattle was then used to determine a correlation between breed and both quality and yield grades (R Studio version 4.1.1). The major breeds in the Guelph population were Angus and Limousin, and Angus and Simmental were the major breeds in the Lacombe population.

Where the effects of ageing for either 7 or 21 days were included in the analysis, the data were analyzed as a split plot, with all meat quality and collagen measures determined using the main plot with animals as the experimental units, while steak portions were the experimental units at the subplot level to examine the effect of post-mortem ageing on collagen solubility. To evaluate the effect of post-mortem aging on collagen solubility, the non-normally distributed collagen solubility data was transformed using a Box-Cox transformation and evaluated using a paired parametric test. The untransformed data was also analyzed with a non-parametric Wilcoxon test (R Studio version 4.1.1).

Data were then analyzed using stepwise multiple linear regression techniques to determine the factors affecting peak shear force values. Factors considered for multiple linear

regression were all those measured, including: final live weight on farm, hot carcass weight, dressing percentage, longissimus thoracis (LT) area (cm^2), back fat (mm), yield grade code, quality grade code, marbling score, LT percent lean, LT percent fat, LT percent bone, LT percent body fat, LT percent subcutaneous fat, LT percent intermuscular fat, LT intramuscular fat, LT L*, LT a*, LT b*, LT chroma, LT hue, pH (taken between 120 and 144 hours post-mortem), percentage of cooking loss, total collagen at 7 days post-mortem (dpm), total collagen at 21 dpm, total collagen from tryptic digesta, total collagen from the pyridinoline assay, percentage of soluble collagen from the sum of soluble and insoluble collagen at 7 dpm, percent soluble collagen from the sum of soluble and insoluble collagen at 21 dpm, percent soluble from total collagen from intramuscular connective tissue (IMCT) at 7 dpm, percent soluble from total collagen from IMCT at 21 dpm, Ehrlich's chromogen concentration (mol/mol collagen), and pyridinoline concentration (mol/mol collagen). Factors were included in the model after all identifiable factors were tested individually using a simple linear regression analysis (R Studio version 4.1.1). Variables that were significantly correlated ($P < 0.05$) according to Pearson correlation coefficient analysis were evaluated for their individual significance to the estimation of shear force, and the less significant variable was excluded from the model. Multiple regression analyses of shear force were performed with the stepwise selection functions in R studio software to determine the formula for predicting shear force.

Lacombe data were divided into 2017 and 2018 experiments as each cohort of data had different experimental designs. The 2017 cohort was a mix of calves and yearlings that were treated with five different steroid combinations plus a control group as previously described. The 2018 cohort was steer calves only, with 30 calves receiving a terminal implant, and 31 receiving no implant. The GLM of R studio software (version 4.1.1) was used to analyze the data in a split

plot design. Steroid use was a source of variation in the whole plot where individual animals were the experimental units, and measures of meat quality and collagen characteristics were examined at this level. Post-mortem aging and its effect on collagen solubility, as well as its interaction with the use of steroidal implants and measures of meat quality and collagen characteristics was a source of variation in the subplot with steaks being the experimental units. Least squares means were compared for the different steroid treatments, where differences were considered significant at $P < 0.05$ using Tukey's HSD from the agricolae package (version 1.3-5). An ANOVA test was performed to examine the effect of post-mortem aging and steroids on collagen solubility, and the interaction of steroidal implants and post-mortem aging in a split plot design. In this split plot design, the steak was the experimental unit for the steroidal treatment at the main plot level, and individual steak subsamples were the experimental units of the subplot for collagen solubility over time.

Factors were included in the model for the multiple linear regression equation of shear force after all identifiable factors were tested for significance using simple linear regression analysis (R Studio version 4.1.1). Factors considered for the multiple linear regression equation of shear force included: age at slaughter (days), steroid treatment, moisture percentage of the LT, fat percentage of the LT, protein percentage of the LT, percentage of cooking loss of the LT, cooking time (sec/g), carcass temperature at 45 minutes post-mortem, temperature at 72 hours post-mortem, pH at 45 minutes post-mortem, pH at 72 hours post-mortem, LT L*, LT a*, LT b*, LT chroma, LT hue, total collagen from tryptic digesta, total collagen from IMCT, percentage of soluble collagen at 7 dpm, percentage of soluble collagen at 21 dpm, Ehrlich's chromogen concentration (mol/mol collagen), and concentration of pyridinoline (mol/mol collagen). Stepwise selection functions were then used to determine the formula for predicting shear force

in the Lacombe samples. When variables were significantly correlated ($P < 0.05$), the variable that was determined to be less significant to shear force was excluded from the model via the stepwise elimination function for the multiple linear regression analysis (R Studio version 4.1.1).

2.14 Genetic analyses: heritability, correlations, and genome-wide association study

The following factors were tested for significance using R studio (version 4.1.1) and SAS to build the model: slaughter batch, days between birth and date of slaughter, hot carcass weight, breed composition, muscle type, and steroid treatment. The final animal model contained breed composition of Angus, Charolais, Simmental, and other that was the sum of fractions of other minor breeds as covariates, slaughter batch as the fixed effect, and slaughter age as a covariate.

GWAS was performed using a single-step genomic best linear unbiased prediction (ssGBLUP) which combines phenotypic and genotypic data in a single step (Mitzal et al. 2009, Christensen and Lund 2010, Wang et al. 2012). The animal model for the analysis is presented as follows:

$$\boldsymbol{Y} = \boldsymbol{X}b + \boldsymbol{Z}a + e$$

where Y is the vector of phenotypic observations (before and after aging collagen solubility, total collagen, Ehrlich's chromogen and pyridinoline), b is the vector of fixed effects (breed composition of Angus, Charolais, Simmental, and other that was the sum of fractions of other minor breeds, slaughter batch, and age at slaughter), a is the vector of additive genetic effects, e is the vector of residual errors, and X and Z are the incidence matrices of b and a respectively. Assumptions included in the model were $a \sim N(0, H\sigma_a^2)$, where H is the relationship matrix combining the genetic matrix G and the numerator matrix A, and σ_a^2 is the additive genetic variance, and $e \sim N(0, I\sigma_e^2)$, where I is the identity matrix and σ_e^2 is the residual variance. As

described by Aguilar et al. (2010) the inverse of the H matrix, which combines genomic and pedigree information, is as follows:

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

where A^{-1} is a numerator pedigree relationship matrix for all animals, A_{22}^{-1} describes the inverse numerator relationship matrix of all animals with known genotypes and phenotypes, and G^{-1} is the inverse of the genomic relationship matrix constructed using an iterative procedure whereby each SNP effect is weighted through its expected variance according to the methods described by VanRaden (2008) as follows:

$$G = ZDZ'q$$

Here, Z is the SNP incidence matrix with values of 0,1, or 2 (with 5 indicating a missing value, which accounted for 0.53%) adjusted for allele frequency, where 0 represents a homozygote at the locus, 1 defines the heterozygote, and 2 the other homozygote; D is the diagonal matrix with the inverse of expected SNP variance, and q is the weighting factor for SNP variances. The program used disposes of any missing genotypes during quality control, prior to completing the analysis. The weighting factor was used according to Vitezica et al. (2011), where the average diagonal of G was ensured to be close to that of A_{22}. Only the genomic matrix G was used for the study, as pedigree was not available. As ssGBLUP uses an H matrix, a pseudo pedigree was created to describe the sires and dams, and weighted at 1%, while 99% of the analysis was based on the G matrix, effectively running the ssGBLUP on the G matrix alone, in order to avoid the confounding effects of the missing pedigree data (Bradford et al. 2019).

Bayesian analysis with Gibbs sampling was used to estimate the heritability (h^2) of the 5 collagen traits, with each run as a single trait analysis using the model defined above. The

gibbs2f90 program, which is part of the BLUPF90 suite of programs, was used to estimate the heritability of each trait. Heritability was defined as $\sigma_a^2/\sigma_a^2+\sigma_u^2$, where σ_a^2 is the additive genetic variance and σ_u^2 is the environmental variance, both of which were estimated by the Gibbs sampling program. Genetic and phenotypic correlations were then estimated using a two-trait (bivariate) analysis using the Gibbs sampling program. The bivariate animal model is described below:

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

in with y_1 and y_2 are the vectors of the phenotypic values for the two traits under analysis, b_1 and b_2 are the vectors of a population mean and fixed effects for trait 1 and trait 2 as previously defined (breed composition of Angus, Charolais, Simmental, and other that was the sum of the fractions of other minor breeds as covariates, slaughter batch as the fixed effect, and a linear covariate of the individual animal's age at slaughter), a_1 and a_2 are vectors of the random additive genetic effects for traits 1 and 2 respectively, e_1 and e_2 are vectors of the random residual errors of the two traits, and $X_1,\ X_2,\ Z_1,\ Z_2$ are design matrices for $b_1,\ b_2,\ a_1,\ a_2$ respectively. Multivariate normal distributions were assumed for the random vector a with means equal to 0, leading to E(y) = X_b. The variance-covariance matrix for the random effects is described below:

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

where $\sigma_{a_1}^2$ and $\sigma_{a_1}^2$ are the additive genetic variance for traits 1 and 2 respectively and $\sigma_{a_1a_2}$ is the covariance between the two traits; H is the additive genetic relationship matrix H that was previously described; $\sigma_{e_1}^2$ and $\sigma_{e_2}^2$ are the residual variances for traits 1 and 2 respectively and

$\sigma_{e_1e_2}$ is the residual covariance between the two traits; and I_{n_e} is the identity matrix with dimensions $n_e \times n_e$, where n_e is the number of animals with records. Additive variance and covariance for genetic correlation and phenotypic variance and covariance for phenotypic correlation were estimated alongside heritability, therefore heritability was estimated prior to genetic and phenotypic correlations.

The iterative process described by Wang et al. (2014) was used to estimate the SNP effects for the weighted single step GWAS. In the initial run as step 1) $D = I$, then 2) the G matrix was calculated using the formula $G = ZDZ'q$, with the weighting factor q set to 0 for the first time; 3) the genomic estimated breeding values (GEBVs) were calculated using the single step genomic best linear unbiased prediction; 4) GEBVs were converted to SNP effects using the equation $\hat{u} = qDZ'(ZDZ'q)^{-1}\hat{a}$, where $\hat{u}$ is the vector of the SNP effect and $\hat{a}$ is the GEBV for the genotyped animals; 5) the weight of each SNP was calculated using $d_i = \hat{u}_i^2 2p_i(1-p_i)$ where i is the i-th SNP; 6) the SNP weights were normalized to keep the total genetic variance constant using a ratio of traces of the previous D matrix and the updated matrix as follows:

$$D_{(t+1)} = \frac{tr(D_{(0)})}{tr(D_{(t+1)})} D_{(t+1)}$$

The process was then looped once through step 4, which increased the weight of SNPs with large effects and decreased the weights of SNPs with small effects without recalculating the animal effects.

The results of the GWAS were reported as genetic variance explained by a sliding window of 10 consecutive SNPs, where SNP windows that were able to account for 1% or more of the total additive genetic variance in the sample population were considered significant. SNP windows were used instead of individual SNPs as quantitative trait loci (QTL) are usually

surrounded by multiple SNPs that are inherited together due to their proximity to the locus, putting the QTL in linkage disequilibrium with the surrounding SNPs (Habier et al. 2011).

2.15 Functional enrichment analysis

Sliding SNP windows of 10 consecutive SNPs that accounted for 1% or more of the genetic variation were used to search for nearby candidate genes (500 Kb both up and downstream of the window) that may influence the phenotypic traits of interest. 500 Kb was used both up and downstream of the SNP to replicate the methods of Lei (2019) in order to more accurately evaluate the validity of previous research which used the data from the 137 steers from Kinsella. Searching for nearby genes also serves to account for genes that are in linkage disequilibrium (LD) with the SNP, as type I errors are high in single marker association tests that fail to account for LD (Vallejo et al. 2017). Linkage disequilibrium has been found to be higher in taurine breeds, and this has been attributed to a stronger bottleneck during breed formation, therefore it is beneficial to account for a wider range of genes in LD with the SNP in a population that is as small and closely related as the one used in this study (Porto-Neto et al. 2014). While LD decay has been shown to occur in small windows (Porto-Neto et al. 2014), previous research has defined up to 20 Mb as being a single QTL region (Vallejo et al. 2017). The Ensembl Biomart database was used to find RefSeq genes to use for gene identification. The Ingenuity Pathway Analysis software (IPA) (Redwood City, CA; www.qiagen.com/ingenuity.com) was used to determine the biological function of the identified genes, as well as visualize biological pathways and mechanisms.

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Chapter 3: Results

3.1 Study 1. Guelph cattle

3.1.1 Guelph meat quality characteristics

Descriptive statistics for the data from the Guelph cattle are shown in Table 3.1, with the estimated genetic breed composition presented in Table 3.2. Results indicated a broad range of yield and quality grade scores, back fat, marbling, intramuscular fat, and measures of collagen solubility, total collagen, and mature collagen crosslinks. The ranges for final and hot carcass weight, LT percent lean, fat percentages in the LT including fat, body fat, subcutaneous and intermuscular fat, WBSF, muscle pH, and muscle colour were limited and low, with low coefficients of variation indicating limited variation in these cattle.

Table 3.1: Descriptive statistics for Guelph animal, carcass and meat quality data

	n	Minimum	Maximum	Mean	Coefficient of Variation
Final live weight on farm (kg)	49	664	930.5	785.97	0.07
Hot carcass weight (kg)	49	407.60	550.03	464.10	0.07
Dressing percentage (%)	49	56.46	62.57	59.06	0.03
YG Code	49	2	5	3.61	0.24
QG Code	49	1	3	2	0.32
LT Area (cm^2)	49	69.68	126.45	98.04	0.14
Backfat (mm)	49	10.16	40.64	25.26	0.29
Marbling score	49	325	831	549.71	0.25
LT % lean	49	40	60.22	48.26	0.09
LT %fat	49	21.15	43.48	34.66	0.14
LT % bone	49	13.88	19.65	17.08	0.08
LT % body fat	49	9.01	20	13.58	0.17
LT % subcutaneous fat	49	27.03	54.4	44.37	0.12
LT % intermuscular fat	49	32.14	63.96	42.05	0.12
pH	49	5.35	5.5	5.41	0.01
Shear force (kg)	49	2.26	3.924	2.90	0.15
Cooking loss %	49	10.98	23.81	18.61	0.13
Intramuscular fat	49	2.66	12.79	6.78	0.38
LT L*	49	34.62	44.15	38.37	0.06
LT a*	49	17.91	24.51	21.57	0.07
LT b*	49	8.03	12.06	10.02	0.10
LT Chroma	49	19.65	27.06	23.80	0.07
LT Hue	49	21.53	28.33	24.89	0.06

Total collagen at 7 dpm mg/g raw meat	49	2.13	4.87	3.21	0.22
Total collagen at 21 dpm mg/g raw meat	49	2.19	4.22	3.09	0.17
Total collagen from tryptic digesta mg/g raw meat	49	0.86	4.15	2.24	0.31
Total collagen from pyridinoline mg/g raw meat	49	1.95	5.06	2.99	0.21
% soluble collagen at 7 dpm from sum total	49	4.51	34.56	16.82	0.43
% aged soluble collagen of sum total	49	9.65	36.41	19.26	0.28
% soluble at 7 dpm from pyridinoline total	49	2.94	58.40	20.10	0.64
% soluble at 21 dpm from pyridinoline total	49	7.89	58.81	21.19	0.46
Ehrlich chromogen mol/mol collagen	49	0.24	1.03	0.44	0.32
Pyridinoline mol/mol collagen	49	0.08	0.31	0.18	0.20

Estimated genetic breed composition results indicated cattle were 0 to 100% Angus, 0 to 93% Limousin, and up to 51% Simmental, indicating purebred and F1 cross cattle were in the population. The proportions of Charolais, Hereford, Shorthorn, Brown-Swiss, Galloway, Gelbvieh, Holstein, Jersey, and Salers had much lower maximums, however, indicating that these breeds were detected in crossbred cattle only.

Table 3.2: Estimated genetic breed composition of cattle from Guelph study

Breed	Minimum	Maximum	Mean	Standard deviation
Angus	0.00002	0.99988	0.59818	0.26106
Charolais	0.00001	0.07886	0.00922	0.01801
Hereford	0.00001	0.13174	0.01937	0.02988
Shorthorn	0.00001	0.05918	0.00636	0.01311
Brown-Swiss	0.00001	0.07863	0.00527	0.01426
Galloway	0.00001	0.03712	0.00305	0.00696
Gelbvieh	0.00001	0.10863	0.01455	0.02172
Holstein	0.00001	0.04198	0.00608	0.00985
Jersey	0.00001	0.05936	0.00575	0.01331
Limousin	0.00001	0.93909	0.14417	0.27565
Maine-Anjou	0.00001	0.11542	0.00906	0.02114
Salers	0.00001	0.11951	0.00893	0.02175
Simmental	0.00001	0.51109	0.17001	0.15006

There were no differences in animal final live weight, hot carcass weight, or dressing percentage due to quality grade ($P > 0.05$), although the decrease in dressing percentage of Canada Prime carcasses when compared to Canada AA carcasses approached significance ($P = 0.1$) (Table 3.3). Yield grade was greatest in Canada Prime and AAA carcasses with Canada AAA carcasses having a smaller mean LT area than Canada AA carcasses ($P < 0.05$) (Table 3.1.3). Mean backfat score was higher in Canada Prime and AAA than Canada AA muscle, and marbling scores were highest in Canada Prime, then Canada AAA and then Canada AA (Table 3.3).

Similar results were obtained using confidence interval comparisons, with no differences between Canada AA and the other grades for final live weight or hot carcass weight, but differences were observed in the other carcass characteristics measured (Table 3.4). Again, Canada AAA carcasses had the lowest mean dressing percentage, while Canada AA carcasses had the lowest mean yield grade, highest LT area, lowest back fat depth and marbling score (Table 3.3).

Table 3.3: Differences in least squares means with standard error (SE) for carcass quality traits due to quality grade according to Tukey's HSD

	Prime (n = 10)	AAA (n = 29)	AA (n = 10)	p-value
Final live weight on farm kg	800.20 (17.14)	785.59 (10.07)	772.85 (17.14)	0.26
Hot carcass weight, (kg)	472.60 (10.32)	460.70 (6.06)	465.47 (10.32)	0.63
Dressing percentage, (%)	59.08 (0.51)	58.64 (0.30)	60.26 (0.51)	0.10
YG Code	4.10^{a} (0.27)	3.76^{a} (0.16)	2.70^{b} (0.27)	<0.01*
LT area cm^2	97.42^{ab} (4.32)	94.30^{b} (2.53)	109.48^{a} (4.32)	0.05*
Backfat mm	29.97^{a} (2.36)	25.72^{a} (1.38)	19.20^{b} (2.36)	<0.01*
Marbling score[2]	768.20^{a} (43.32)	528.28^{b} (25.44)	393.40^{c} (43.32)	<0.01*

[a,b,c] Means with different superscripts in the same row are different at $P < 0.05$.
* indicates a significant p-value of <0.05.
[2]Marbling score ranges from 100 (no intramuscular fat) to 1190 (extreme amounts of intramuscular fat)

Table 3.4: Confidence interval comparison of Canada AA carcass measurement means with standard deviation (SD) to those of Canada AAA and Prime carcasses for carcass quality grade

	Prime (n = 10)	AAA (n = 29)	AA (n = 10)
Final live weight on farm (kg)	800.20 (59.90)	785.59 (52.57)	772.85 (55.36)
Hot carcass weight (kg)	472.60 (35.16)	460.70 (32.40)	465.47 (32.50)
Dressing %	59.08 (1.47)	58.64 (1.36)*	60.26 (1.92)
YG Code	4.10 (0.57)*	3.76 (0.79)*	2.70 (0.67)
LT area (cm^2)	97.42 (14.44)*	94.30 (12.63)*	109.48 (9.87)
Backfat (mm)	29.97 (5.55)*	25.72 (7.11)*	19.20 (6.49)
Marbling score[2]	768.20 (33.29)*	528.28 (71.51)*	393.40 (37.62)

* Means with an asterisk are significantly different ($P < 0.05$) from the Canada AA carcass mean within the row (95% confidence intervals adjusted for effect size at https://www.campbellcollaboration.org/research-resources/effect-size-calculator.html)
[2]Marbling score ranges from 100 (no intramuscular fat) to 1190 (extreme amounts of intramuscular fat)

The percentage of lean muscle tissue was highest in Canada AA carcasses with Canada Prime and Canada AA carcasses having similar percentages of lean, while the percentage of fat was lowest in the Canada AA carcasses (Table 3.5). There were no significant differences between the quality grades for bone mass percentage of the rib section, subcutaneous fat, intermuscular fat, or body fat, nor for LT pH or cooking loss ($P > 0.05$). Intramuscular fat decreased as quality grade increased ($P < 0.0001$). Shear force was higher in beef from Canada AA carcasses than in that from Canada Prime carcasses, while intramuscular fat was highest in Canada Prime and lowest in Canada AA muscles ($P < 0.05$) (Table 3.5).

These findings were consistent with those of the confidence interval results, with Canada AA carcasses being leaner (higher percentage lean score, lower percentage fat, lower intramuscular fat score) than Canada Prime and AAA steers (Table 3.6). Canada Prime also had a lower mean shear force value than Canada AA steers, though AA and AAA did not significantly differ in mean shear force values (Table 3.6).

Table 3.5: Differences in least squares means with standard error (SE) for LT dissection composition values due to quality grade according to Tukey's HSD

	Prime (n = 10)	AAA (n = 29)	AA (n = 10)	p-value
LT % lean	46.27[a] (1.32)	47.21[a] (0.78)	53.29[b] (1.32)	<0.01*
LT % fat	37.22[a] (1.51)	35.66[a] (0.88)	29.22[b] (1.51)	<0.01*
LT % bone	16.51 (0.46)	17.13 (0.27)	17.49 (0.46)	0.13
LT % body fat	12.50 (0.73)	13.64 (0.43)	14.46 (0.73)	0.05
LT % subcutaneous fat	44.15 (1.69)	44.02 (0.99)	45.63 (1.69)	0.54
LT % intermuscular fat	43.36 (1.65)	42.34 (0.97)	39.92 (1.65)	0.14
pH	5.40 (0.01)	5.41 (0.01)	5.42 (0.01)	0.43
Shear force (kg)	2.62[a] (0.14)	2.93[ab] (0.08)	3.07[b] (0.14)	0.01*
Cooking loss %	17.23 (0.78)	19.28 (0.46)	18.05 (0.78)	0.46
IMF	10.16[a] (0.83)	6.46[b] (0.49)	4.33[c] (0.83)	<0.01*

[a,b,c] Means with different superscripts in the same row are different at $P < 0.05$.
* indicates a significant p-value of <0.05.

Table 3.6: Confidence interval comparison of Canada AA carcass LT dissection composition values with standard deviation (SD) to those of Canada AAA and Prime

	Prime (n = 10)	AAA (n = 29)	AA (n = 10)
LT % lean	46.27 (1.97)*	47.21 (3.34)*	53.29 (4.28)
LT % fat	37.22 (2.80)*	35.66 (3.85)*	29.22 (4.84)
LT % bone	16.51 (1.46)	17.13 (1.45)	17.49 (1.41)
LT % body fat	12.50 (2.30)	13.64 (2.16)	14.46 (2.49)
LT % subcutaneous fat	44.15 (5.07)	44.02 (5.48)	45.63 (5.51)
LT % intermuscular fat	43.36 (3.96)	42.34 (5.48)	39.92 (5.44)
pH	5.40 (0.03)	5.41 (0.05)	5.42 (0.05)
Shear force kg	2.62 (0.24)*	2.93 (0.44)	3.07 (0.42)
Cooking loss %	17.23 (2.74)	19.28 (2.22)	18.05 (2.41)
IMF	10.16 (2.29)*	6.46 (1.76)*	4.33 (1.56)

* Means with an asterisk are significantly different ($P < 0.05$) from the Canada AA carcass mean within the row (95% confidence intervals adjusted for effect size at https://www.campbellcollaboration.org/research-resources/effect-size-calculator.html)

Canada Prime carcasses had higher L* values than Canada AA carcasses, although Canada AAA did not differ from Canada AA (Table 3.7). Mean hue values for Canada Prime carcasses were greater than those from Canada AA (Table 3.7). Confidence interval testing results reflected similar results (Table 3.8). Measurements of a*, b*, and chroma were similar for all grades ($P > 0.05$).

Table 3.7: Differences in least squares means with standard error (SE) for LT colour values due to quality grade according to Tukey's HSD

	Prime (n = 10)	AAA (n = 29)	AA (n = 10)	p-value
LT L*	40.15[a] (0.69)	38.25[b] (0.41)	36.93[b] (0.69)	<0.01*
LT a*	21.08 (0.47)	21.57 (0.28)	22.06 (0.47)	0.14
LT b*	10.19 (0.31)	10.03 (0.18)	9.85 (0.31)	0.43
LT Chroma	23.44 (0.53)	23.81 (0.31)	24.16 (0.53)	0.33
LT Hue	25.82[a] (0.48)	24.87[ab] (0.28)	24.03[b] (0.48)	0.01*

[a,b] Means with different superscripts in the same row are different at $P < 0.05$.
* indicates a significant p-value of <0.05.

Table 3.8: Canada AA LT colour measurements with standard deviation (SD) compared to those of Canada Prime and AAA using confidence interval testing

	Prime (n = 10)	AAA (n = 29)	AA (n = 10)
LT L*	40.15 (2.43)*	38.25 (1.87)	36.93 (1.71)
LT a*	21.08 (1.76)	21.57 (1.43)	22.06 (1.40)
LT b*	10.19 (0.82)	10.03 (1.08)	9.85 (0.84)
LT Chroma	23.44 (1.85)	23.81 (1.69)	24.16 (1.53)
LT Hue	25.82 (1.46)*	24.87 (1.43)	24.03 (1.45)

* Means with an asterisk are significantly different ($P < 0.05$) from the Canada AA carcass mean within the row (95% confidence intervals adjusted for effect size at https://www.campbellcollaboration.org/research-resources/effect-size-calculator.html)

Collagen characteristics at 7 dpm differed significantly between quality grades ($P < 0.05$) (Table 3.9). Canada Prime and AAA had a lower percent of soluble collagen in the 7 dpm samples, both when total collagen was a sum of soluble and insoluble and when determined from IMCT, and Canada AAA had the lowest percentage of collagen solubility, although it was not significantly different from Canada Prime in either case (Table 3.9). There was no significant difference between quality grades when total collagen was measured as a sum of soluble and insoluble collagen in raw meat at either 7 days post-mortem, and total collagen from the tryptic digesta or the pyridinoline assay did not significantly differ between quality grades either (Table 3.9). While the p-value indicated a significant difference between quality grades for total collagen at 21 days post-mortem for total collagen as a sum of soluble and insoluble collagen ($P = 0.04$), the Tukey's Honestly Significant Difference test did not find a difference between

grades. There was no significant difference between mean total collagen in aged samples, and collagen solubility as a percentage of total collagen from the pyridinoline assay did not differ between quality grades means for the aged samples ($P > 0.05$). Ehrlich's chromogen and pyridinoline concentrations did not differ due to quality grade (Table 3.9).

At a 95% confidence interval, total collagen as a sum of soluble and insoluble collagen from the 7 dpm samples was lower in Canada AAA carcasses than in those from Canada AA, though total collagen between Canada Prime and Canada AA did not significantly differ (Table 3.10). The sum of soluble and insoluble collagen in 21 dpm samples was lower in Canada AA carcasses than in those from the Canada Prime and AAA. The mean 7 dpm collagen solubility as a percent of total collagen both as a sum of soluble and insoluble collagen and calculated from the pyridinoline assay was only found to differ between Canada AAA and AA, with Prime being similar to Canada AA carcasses (Table 3.10).

Table 3.9: Differences in least squares means with standard error (SE) for LT collagen characteristics due to quality grade according to Tukey's HSD

	Prime (n = 10)	AAA (n = 29)	AA (n = 10)	p-value
Total day 7 collagen, mg/g raw meat	3.39 (0.23)	3.02 (0.13)	3.56 (0.23)	0.59
Total day 21 collagen, mg/g raw meat	3.23 (0.16)	3.15 (0.10)	2.76 (0.16)	0.04*
Total collagen from tryptic digesta, mg/g raw meat	2.19 (0.22)	2.38 (0.13)	1.90 (0.22)	0.37
Total collagen from pyridinoline mg/g raw meat	3.27 (0.20)	2.98 (0.12)	2.73 (0.20)	0.05
% soluble collagen in sum total day 7	16.52^{ab} (2.28)	14.91^{b} (1.34)	22.64^{a} (2.28)	0.06
% soluble collagen in sum total day 21	17.86 (1.74)	20.90 (1.02)	19.18 (1.74)	0.22
% of 7 dpm soluble collagen (from pyridinoline assay total)	18.98^{ab} (4.08)	16.76^{b} (2.40)	30.89^{a} (4.08)	0.04*
% of 21 dpm soluble collagen (from pyridinoline assay total)	19.18 (3.10)	21.54 (1.82)	22.16 (3.10)	0.50
Ehrlich chromogen mol/mol collagen	0.43 (0.05)	0.45 (0.03)	0.43 (0.05)	0.99
Pyridinoline mol/mol collagen	0.17 (0.01)	0.19 (0.01)	0.17 (0.01)	0.92

[a,b] Means with different superscripts in the same row are different at $P < 0.05$.
* indicates a significant p-value of <0.05.

Table 3.10: Comparison of collagen characteristics means with standard deviation (SD) of Canada AA to those of Prime and Canada AAA using confidence interval testing

	Prime (n=10)	AAA (n=29)	AA (n=10)
Total day 7 collagen mg/g raw meat (sum of soluble and insoluble)	3.39 (0.74)	3.02 (0.67)*	3.56 (0.70)
Total day 21 collagen mg/g raw meat (sum of soluble and insoluble)	3.23 (0.44)*	3.15 (0.56)*	2.76 (0.33)
Total collagen from tryptic digesta mg/g raw meat	2.19 (0.63)	2.38 (0.74)	1.90 (0.54)
Total collagen from pyridinoline mg/g raw meat	3.27 (0.70)	2.98 (0.67)	2.73 (0.45)
% of 7 dpm soluble collagen in (sum) total	16.52 (7.33)	14.91 (5.85)*	22.64 (8.29)
% of 7 dpm soluble collagen (from pyridinoline assay total)	18.98 (11.38)	16.76 (10.49)*	30.89 (15.82)
% of 21 dpm soluble collagen in (sum) total	17.86 (5.42)	19.18 (5.69)	20.90 (5.15)
% of 21 dpm soluble collagen in pyridinoline total	19.18 (9.58)	21.54 (10.46)	22.16 (8.68)
Ehrlich chromogen mol/mol collagen	0.43 (0.11)	0.45 (0.16)	0.43 (0.14)
Pyridinoline mol/mol collagen	0.17 (0.05)	0.19 (0.03)	0.17 (0.14)

* Means with an asterisk are significantly ($P < 0.05$) different from the Canada AA carcass mean within the row (95% confidence intervals adjusted for effect size at https://www.campbellcollaboration.org/research-resources/effect-size-calculator.html)

When the data were putatively categorized by yield grade, there were no significant differences between yield grades for final animal weight or hot carcass. Despite the P value being significant, the Tukey's HSD analysis found no significant difference between the LT area of different yield grades. Mean dressing percentage in yield grade 2 was higher than that in yield grade 4, and quality grade was highest in yield grades 2 and 3 (Table 3.11). Backfat and marbling scores increased as yield grade increased, with yield grades 2 and 3 having the lowest mean back fat thickness (Table 3.11). When compared to yield grade 5 however, dressing percentage was not significantly different between carcasses with a yield grade of 5 and any other yield grade (Table 3.12). The LT area of yield grade 5 carcasses was smaller than for grades 2 and 3, although it was not different from grade 4, while the backfat thickness of yield grade 5 carcasses was higher than any other grade (Table 3.12). Marbling score was also higher

in yield grade 5 steers than those in yield grades 2 and 3, but not steer carcasses of yield grade 4 (Table 3.12).

Table 3.11: Differences in least squares means with standard error (SE) for LT carcass quality traits due to yield grade according to Tukey's HSD

	2 (n=6)	3 (n=13)	4 (n=24)	5 (n=6)	p-value
Final live weight kg	758.83 (22.13)	791.96 (15.03)	793.42 (11.06)	770.33 (22.13)	0.64
Hot carcass weight kg	460.14 (13.32)	468.61 (9.05)	465.61 (6.66)	452.25 (13.32)	0.68
Dressing %	60.68^{a} (0.66)	59.20^{ab} (0.45)	58.68^{b} (0.33)	58.71^{ab} (0.66)	0.01*
QG Code	2.67^{a} (0.26)	2.31^{a} (0.18)	1.75^{b} (0.13)	1.67^{b} (0.26)	<0.01*
LT Area cm^2	107.54 (5.57)	104.52 (3.79)	93.76 (2.79)	91.61 (5.57)	<0.01*
Backfat mm	14.73^{c} (3.04)	19.85^{c} (2.07)	28.28^{b} (1.52)	35.39^{a} (3.04)	<0.01*
Marbling score	423.17^{b} (55.93)	499.85^{ab} (38.00)	583.63^{a} (27.97)	648.67^{a} (55.09)	<0.01*

^{a,b,c} Means with different superscripts in the same row are different at $P < 0.05$.
* indicates a significant p-value of <0.05.

Table 3.12: Comparison using confidence intervals of means with standard deviation (SD) of yield grade 5 carcass quality traits to those of grades 2, 3, and 4

	2 (n=6)	3 (n=13)	4 (n=24)	5 (n=6)
Final live weight kg	758.83 (50.87)	791.96 (61.06)	793.42 (54.52)	770.33 (38.19)
Hot carcass weight kg	460.14 (28.49)	468.61 (34.20)	465.61 (35.20)	452.25 (25.55)
Dressing %	60.68 (1.79)	59.20 (1.48)	58.68 (1.49)	58.71 (1.46)
QG Code	2.67 (0.52)*	2.31 (0.63)	1.75 (0.53)	1.67 (0.52)
LT Area cm^2	107.53 (8.28)*	104.52 (13.48)*	93.76 (13.90)	91.61 (6.31)
Backfat mm	14.73 (3.19)*	19.85 (4.68)*	28.28 (4.45)*	35.39 (1.75)
Marbling score	423.17 (91.37)*	499.85 (133.34)*	583.63 (126.41)	648.67 (112.84)

* Means with an asterisk are significantly ($P < 0.05$) different from the yield grade 5 AA carcass mean within the row (95% confidence intervals adjusted for effect size at https://www.campbellcollaboration.org/research-resources/effect-size-calculator.html)

When analyzed for differences across yield grade, the percent of lean muscle tissue was highest in yield grade 2 and lowest in yield grades 4 and 5 (Table 3.13). Fat percentages increased as yield grade increased (Table 3.13). The bone mass of the LT expressed as a percentage was lower in yield grade 5 than in yield grade 2 and 3 ($P < 0.001$), while body fat was higher in yield grade 2 than in all other yield grades ($P = 0.0015$). The percentage of subcutaneous fat and intermuscular fat, mean pH, percentage of sample weight lost in cooking,

and shear force (kg) did not differ between yield grades (P > 0.05), and mean IMF was not significantly different between yield grades (P > 0.05). Intramuscular fat was lower in yield grades 2 and 3 than in grades 4 and 5, but the Tukey's HSD test did not identify a significant difference in the means between the grades (Table 3.13).

When analyzed using confidence interval comparisons, carcasses with a yield grade of 5 had lower lean and bone percentages and higher fat percentages than grades 2, 3, and 4, and significantly lower body fat percentages than carcasses with a yield grade of 2, although the body fat percentage values of yield grade 3 and 4 carcasses were similar to those of yield grade 5 (Table 3.14). No differences were observed for percentage of subcutaneous fat, intermuscular fat, or pH, WBSF, cooking loss or percentage IMF (P > 0.05) (Table 3.14).

Table 3.13: Differences in least squares means with standard error (SE) for LT dissection values due to yield grade according to Tukey's HSD

	2 (n=6)	3 (n=13)	4 (n=24)	5 (n=6)	p-value
LT % lean	54.97^{a} (1.70)	50.06^{b} (1.16)	46.73^{c} (0.85)	43.78^{c} (1.70)	<0.01*
LT % fat	27.03^{d} (1.94)	32.35^{c} (1.32)	36.29^{b} (0.97)	40.79^{a} (1.94)	<0.01*
LT % bone	18.01^{a} (0.59)	17.59^{a} (0.40)	16.98^{ab} (0.30)	15.44^{b} (0.59)	<0.01*
LT % bodyfat	16.43^{a} (0.94)	13.62^{b} (0.64)	13.14^{b} (0.47)	12.38^{b} (0.94)	<0.01*
LT % subfat	43.43 (2.18)	44.26 (1.48)	44.35 (1.09)	45.67 (2.18)	0.52
LT % intermuscular fat	40.14 (2.13)	42.13 (1.45)	42.52 (1.07)	41.95 (2.13)	0.50
pH	5.40 (0.02)	5.42 (0.01)	5.42 (0.01)	5.38 (0.02)	0.49
Shear force kg	2.99 (0.17)	2.97 (0.12)	2.82 (0.09)	2.97 (0.17)	0.54
Cooking loss %	19.19 (1.01)	18.07 (0.69)	18.53 (0.50)	19.55 (1.01)	0.70
IMF	5.40 (1.07)	5.63 (0.73)	7.48 (0.54)	7.82 (1.07)	0.02*

^{a,b,c} Means with different superscripts in the same row are different at P < 0.05.
* indicates a significant p-value of <0.05.

Table 3.14: Comparison of LT mean with standard deviation (SD) dissection values of yield grade 5 steer carcasses to those of yield grades 2, 3, and 4

	2 (n=6)	3 (n=13)	4 (n=24)	5 (n=6)
LT % lean	54.97 (3.19)*	50.06 (3.50)*	46.73 (2.26)*	43.78 (2.53)
LT % fat	27.03 (3.51)*	32.35 (3.95)*	36.29 (2.21)*	40.79 (1.98)
LT % bone	18.01 (1.36)*	17.59 (1.21)*	16.98 (1.32)*	15.44 (1.36)
LT % bodyfat	16.43 (2.11)*	13.62 (1.65)	13.14 (2.28)	12.38 (1.86)
LT % subfat	43.43 (6.20)	44.26 (5.07)	44.35 (5.99)	45.67 (2.05)
LT % intermuscular fat	40.14 (5.98)	42.13 (4.92)	42.52 (5.93)	42.00 (1.12)
pH	5.40 (0.04)	5.42 (0.04)	5.42 (0.05)	5.38 (0.03)
Shear force kg	2.99 (0.52)	2.97 (0.37)	2.82 (0.46)	2.97 (0.34)
Cooking loss %	19.19 (2.30)	18.07 (1.99)	18.53 (2.78)	19.55 (2.41)
IMF (%)	5.40 (1.70)	5.63 (2.46)	7.48 (2.72)	7.82 (2.37)

* Means with an asterisk are significantly different ($P < 0.05$) from the yield grade 5 AA carcass mean within the row (95% confidence intervals adjusted for effect size at https://www.campbellcollaboration.org/research-resources/effect-size-calculator.html)

There were no meaningful differences of mean L*, a*, or chroma values between yield grades. The yield grade 5 LT mean b* and hue values were greater than that of yield grade 2 LT when assessed using least squares means differences ($P < 0.05$) (Table 3.15), but when tested using confidence intervals, carcasses with a yield grade of 5 had a significantly higher b* value than all other grades while the LT hue means from yield grades 2 and 3 were lower than those of yield grade 5 (Table 3.16). There was no meaningful difference in mean hue value between yield grades 4 and 5 (Table. 3.16).

Table 3.15: Differences in least squares means with standard error (SE) for LT colour values due to yield grade according to Tukey's HSD

	2 (n=6)	3 (n=13)	4 (n=24)	5 (n=6)	p-value
LT L*	37.01 (0.89)	38.33 (0.61)	38.45 (0.45)	39.44 (0.89)	0.08.
LT a*	21.85 (0.61)	21.55 (0.42)	21.30 (0.31)	22.39 (0.61)	0.84
LT b*	9.48^{b} (0.40)	9.95^{ab} (0.27)	9.95^{ab} (0.20)	11.04^{a} (0.40)	0.02*
LT Chroma	23.83 (0.68)	23.75 (0.46)	23.53 (0.34)	24.97 (0.68)	0.46
LT Hue	23.43^{b} (0.62)	24.70^{ab} (0.42)	25.03^{ab} (0.31)	26.24^{a} (0.62)	<0.01*

^{a,b} Means with different superscripts in the same row are different at $P < 0.05$.
* indicates a significant p-value of <0.05.

Table 3.16: Comparison of yield grade 5 LT colour values with standard deviation (SD) to those of yield grades 2, 3, and 4

	2 (n=6)	3 (n=13)	4 (n=24)	5 (n=6)
LT L*	37.01 (1.98)*	38.33 (2.02)	38.45 (38.45)	39.44 (2.37)
LT a*	21.85 (1.75)	21.54 (1.22)	21.30 (1.62)	22.39 (1.24)
LT b*	9.48 (1.04)*	9.95 (0.95)*	9.95 (0.92)*	11.04 (0.62)
LT Chroma	23.83 (1.93)	23.75 (1.44)	23.53 (23.53)	24.97 (1.34)
LT Hue	23.43 (1.58)*	24.70 (1.33)*	25.03 (1.46)	26.24 (0.85)

* Means with an asterisk are significantly different ($P < 0.05$) from the yield grade 5 AA carcass mean within the row (95% confidence intervals adjusted for effect size at https://www.campbellcollaboration.org/research-resources/effect-size-calculator.html)

As observed for quality grade, only 7 dpm collagen characteristics differed between yield grades. Yield grade 2 had the highest values for of 7 dpm percentage soluble collagen when total collagen was a sum of soluble and insoluble collagen ($P = 0.0016$), and 7 dpm soluble collagen as a percentage of hydrolyzed IMCT ($P=0.0001$). There was no significant difference between means of collagen characteristics in the 21 dpm samples for any of the yield grades, and total collagen when measured from tryptic digesta and hydrolyzed IMCT, as well as Ehrlich's chromogen and pyridinoline content were similar across all grades (Table 3.17).

Collagen characteristics did not differ significantly between yield grade 5 carcasses and those of yield grades 3 and 4, however there were meaningful differences of means betweel yield grades 5 and 2 for several collagen traits (Table 3.18). The mean total collagen in raw meat at 7 dpm was significantly higher in yield grade 2 than in yield grade 5 carcasses, though the means of yield grades 3 and 4 did not differ from yield grade 5 (Table 3.18). Yield grade 2 carcasses when compared to grade 5 steers had a higher total collagen when expressed as a sum of soluble and insoluble collagen in both 7 dpm and aged samples, higher percent soluble collagen in 7 dpm samples, and a higher percent soluble collagen from total collagen as calculated from IMCT (Table 3.18).

Table 3.17: Differences in least squares means with standard error (SE) for LT collagen characteristics due to yield grade according to Tukey's HSD

	2 (n=6)	3 (n=13)	4 (n=24)	5 (n=6)	p-value
Total 7 dpm collagen (sum of soluble and insoluble mg/g raw meat)	3.73 (0.29)	3.27 (0.20)	3.17 (0.15)	2.70 (0.29)	0.02*
Total 21 dpm collagen (sum of soluble and insoluble mg/g raw meat)	3.25 (0.21)	2.78 (0.14)	3.21 (0.11)	3.11 (0.21)	0.48
Total collagen from tryptic digesta mg/g raw meat	1.94 (0.28)	2.28 (0.19)	2.28 (0.14)	2.30 (0.28)	0.39
Total collagen in pyridinoline mg/g raw meat	2.57 (0.26)	2.82 (0.18)	3.16 (0.13)	3.05 (0.26)	0.05*
% of 7 dpm soluble collagen in (sum) total	25.43[a] (2.95)	16.99[ab] (2.00)	15.62[b] (1.47)	12.60[b] (2.95)	<0.01*
% of 7 dpm soluble collagen in pyridinoline total	39.41[a] (5.27)	19.96[b] (3.58)	17.30[b] (2.63)	12.27[b] (5.27)	<0.01*
% of 21 dpm soluble collagen in (sum) total	23.13 (2.25)	18.60 (1.53)	19.32 (1.12)	16.63 (2.25)	0.10
% of 21 dpm soluble collagen in pyridinoline total	29.47 (4.01)	19.12 (2.72)	21.03 (2.00)	18.02 (4.01)	0.12
Ehrlich chromogen mol/mol collagen	0.44 (0.06)	0.42 (0.04)	0.46 (0.03)	0.43 (0.06)	0.74
Pyridinoline mol/mol collagen	0.19 (0.02)	0.18 (0.01)	0.19 (0.01)	0.18 (0.02)	0.98

[a,b] Means with different superscripts in the same row are different at $P < 0.05$.
* indicates a significant p-value of <0.05.

Table 3.18: Comparison of yield grade 5 carcass means with standard deviation (SD) for collagen characteristics to means from yield grades 2, 3, and 4 muscles

	2 (n=6)	3 (n=13)	4 (n=24)	5 (n=6)
Total 7 dpm collagen (sum of soluble and insoluble)	3.73 (0.85)*	3.27 (0.86)	3.17 (0.57)	2.70 (0.55)
Total 21 dpm collagen (sum of soluble and insoluble)	3.25 (0.52)	2.78 (0.34)	3.21 (0.58)	3.11 (0.33)
Total collagen from tryptic digesta mg/g raw meat	1.94 (0.62)	2.28 (0.56)	2.28 (0.81)	2.30 (0.57)
Total collagen in pyridinoline mg/g raw meat	2.57 (0.38)	2.82 (0.46)	3.16 (0.74)	3.05 (0.66)
% of 7 dpm soluble collagen in (sum) total	25.43 (7.31)*	16.99 (7.67)	15.62 (5.77)	12.60 (5.95)
% of 7 dpm soluble collagen in pyridinoline total	39.41 (18.07)*	19.96 (9.96)	17.30 (9.58)	12.27 (7.40)
% of 21 dpm soluble collagen in (sum) total	23.13 (5.01)	18.60 (4.20)	19.32 (6.00)	16.63 (5.53)
% of 21 dpm soluble collagen in pyridinoline total	29.47 (6.92)*	19.12 (7.49)	21.03 (11.26)	18.02 (7.29)
Ehrlich chromogen mol/mol collagen	0.44 (0.09)	0.42 (0.11)	0.46 (0.15)	0.43 (0.13)
Pyridinoline mol/mol collagen	0.19 (0.02)	0.18 (0.02)	0.19 (0.04)	0.18 (0.03)

* Means with an asterisk are significantly different ($P < 0.05$) from the yield grade 5 AA carcass mean within the row (95% confidence intervals adjusted for effect size at https://www.campbellcollaboration.org/research-resources/effect-size-calculator.html)

The overall breed composition was significantly different between different quality and yield grades (Table 3.19). Angus was positively associated with Canada Prime carcass grades ($P < 0.0001$) and higher yield grades ($P<0.0001$). Charolais was not correlated with quality grade but was inversely related to yield grade, with yield grade decreasing in animals with a higher percentage of Charolais genetics. Limousin breeding was significantly associated with lower quality grades ($P<0.0001$) and yield grades ($P = 0.0013$). Animals with a higher amount of Salers in their breed composition had lower quality grades ($P=0.0221$) and yield grades ($P=0201$). All other breeds that were estimated in the genetic breed composition of the samples were not significantly related to quality grade or yield grade ($P > 0.05$) (Table 3.19).

Table 3.19: Pearson correlation coefficients of quality and yield grade and estimated genetic breed composition, with shaded correlations significant at $P < 0.05$

	Quality grade		Yield grade	
Breed	Correlation	p-value	Correlation	p-value
Angus	-0.62	<0.01	0.62	<0.01
Charolais	0.23	0.12	-0.33	0.02
Hereford	-0.14	0.37	-0.04	0.79
Shorthorn	0.07	0.65	<0.01	0.98
Brown-Swiss	0.19	0.21	-0.21	0.16
Galloway	0.01	0.93	0.08	0.60
Gelbvieh	0.17	0.27	-0.36	0.01
Holstein	0.18	0.22	-0.22	0.15
Jersey	0.17	0.26	-0.16	0.28
Limousin	0.58	<0.01	-0.46	<0.01
Maine-Anjou	-0.23	0.13	0.08	0.61
Salers	0.34	0.02	-0.34	0.02
Simmental	-0.08	0.59	-0.04	0.78

3.1.2. Pearson correlations between collagen and meat quality for Guelph steers

Meat quality traits also had some effect on total collagen. Total collagen in the 7 dpm samples significantly increased as the percentage of lean muscle increased and was inversely correlated with the percentage of fat in the LT and the marbling score. It was also inversely related to LT scores for L* and hue (Table 3.20). Total collagen in the aged samples was not correlated with any of the meat quality traits ($P > 0.05$). Total collagen when determined from tryptic digesta increased with pH and cooking loss ($P < 0.05$) and total collagen from hydrolyzed IMCT as part of the pyridinoline assay increased as backfat increased ($P = 0.02$).

Meat quality traits were not related to the percentage of soluble collagen at 7 dpm, except for LT lean percentage, which increased with collagen solubility, and LT fat and hue, which both decreased with solubility ($P < 0.05$). Excluding this, there were no other significant correlations between any meat quality traits and the percent of 7 dpm or 21 dpm soluble collagen when total collagen was determined as a sum of soluble and insoluble collagen (Table 3.21). 7 dpm collagen solubility when expressed as a percentage of total collagen from isolated IMCT decreased as

backfat, the percentage of fat in the LT, and LT hue increased, and was positively correlated with the percentage of lean muscle tissue in the LT (Table 3.21). Soluble collagen in the aged samples, however, increased as percentages of LT intermuscular fat increased (P = 0.02, r = 0.30) and when subcutaneous fat in the LT decreased (P = 0.024, r = -0.33) when soluble collagen was expressed as a percentage of total collagen from the pyridinoline assay.

There were also some significant Pearson correlations between meat quality and collagen crosslinks. While Ehrlich chromogen content was not related to meat quality traits, the pyridinoline content of the samples significantly increased when hot carcass weight and cooking loss increased and was lower in samples with high values for LT L* and chroma (Table 3.22).

Table 3.20: Pearson correlation coefficients between meat quality and total collagen characteristics

	Total 7 dpm collagen	Total 21 dpm collagen	Tryptic digesta total collagen	Pyridinoline total collagen
Final live weight	-0.05	0.03	0.09	0.01
Hot carcass weight	0.04	<0.01	<0.01	0.05
Dressing %	0.27	-0.06	-0.23	0.09
LT area	0.02	-0.25	-0.05	0.19
Backfat mm	-0.15	0.23	0.06	0.33*
Marbling score	-0.37*	0.28	0.11	0.20
LT % lean	0.29*	-0.08	-0.18	-0.11
LT % fat	-0.31*	0.13	0.14	0.10
LT % bone	0.19	-0.21	0.07	-0.01
LT % bodyfat	0.09	-0.04	0.03	-0.12
LT % subfat	-0.12	-0.09	0.01	0.02
LT % intermuscular fat	0.09	0.11	-0.02	0.03
LT L*	-0.32*	0.09	-0.26	-0.10
LT a*	0.28	0.25	0.12	< -0.01
LT b*	-0.01	0.18	0.04	-0.01
LT chroma	0.22	0.25	0.11	-0.01
LT hue	-0.30*	<-0.01	-0.08	<-0.01
pH	0.06	0.04	0.29*	0.03
Shear force	0.11	0.03	-0.05	0.15
Cooking loss %	0.03	0.20	0.38*	-0.08
Intramuscular fat	-0.27	0.25	-0.17	0.03

* Correlations with an asterisk denote a significant correlation with a P-value < 0.05

Table 3.21: Pearson correlation coefficients between meat quality characteristics and collagen solubility in LT muscle

	7 dpm % soluble collagen from sum total	21 dpm % soluble collagen from sum total	7 dpm % soluble collagen from pyridinoline total	21 dpm % soluble collagen from pyridinoline total
Final live weight	-0.29	0.03	-0.21	0.03
Hot carcass weight	-0.12	0.10	-0.14	0.04
Dressing %	0.21	0.21	0.19	0.06
LT area	0.26	0.03	0.16	-0.11
Backfat mm	-0.24	-0.19	-0.38*	-0.19
Marbling score	-0.20	-0.17	-0.22	-0.08
LT % lean	0.55*	0.22	0.54*	0.19
LT % fat	-0.54*	-0.23	-0.55*	-0.18
LT % bone	0.17	0.12	0.26	0.04
LT % bodyfat	0.23	0.09	0.23	0.08
LT % subcutaneous fat	-0.21	-0.25	-0.27	-0.33*
LT % intermuscular fat	0.11	0.22	0.17	0.30*
LT L*	-0.22	-0.24	-0.22	-0.10
LT a*	0.02	0.25	0.05	0.24
LT b*	-0.22	0.05	-0.21	0.07
LT chroma	-0.04	0.21	-0.01	0.21
LT hue	-0.32*	-0.16	-0.34*	-0.13
pH	0.07	-0.03	-0.06	-0.06
Shear force	0.09	0.11	0.01	-0.02
Cooking loss %	0.02	0.07	0.05	0.16
Intramuscular fat	-0.17	-0.12	-0.18	-0.01

* Correlations with an asterisk denote a significant correlation with a P-value < 0.05

Table 3.22: Pearson correlation coefficients between meat quality characteristics and mature collagen crosslinks

	Ehrlich chromogen mol/mol collagen	Pyridinoline mol/mol collagen
Final live weight	-0.11	0.20
Hot carcass weight	-0.12	0.28*
Dressing %	-0.02	0.23
LT area	-0.15	-0.16
Backfat mm	0.07	0.02
Marbling score	0.64	-0.05
LT % lean	-0.07	-0.07
LT % fat	<0.01	0.12
LT % bone	0.07	-0.21
LT % bodyfat	-0.25	0.06
LT % subfat	-0.09	0.01
LT % intermuscular fat	-0.13	-0.03
LT L*	0.17	-0.34*
LT a*	0.27	0.13
LT b*	-0.20	-0.10
LT chroma	-0.05	0.09
LT hue	-0.18	-0.29*
pH	0.14	0.20
Shear force	-0.20	0.11
Cooking loss %	-0.06	0.47*
Intramuscular fat	-0.21	-0.24

* Correlations with an asterisk denote a significant correlation with a P-value < 0.05

3.1.3. Multiple linear regression of shear force

The multiple regression stepwise selection determined that the formula for predicting shear force (kg) for the Guelph samples included LT a* (P = 0.01975), cooking loss % (P = 0.03462), and intramuscular fat (P = 0.01510). This regression equation explained 22.55% of the variation in shear force.

The regression equation for shear force of the Guelph samples as determined by the stepwise regression analysis is:

Shear force = 1.13060 + 0.07044*(LT a*) + 0.03322*(cooking loss %) – 0.05456*(intramuscular fat).

3.1.4. Collagen solubility over time

Collagen solubility was expressed as a percentage of total collagen when total collagen was determined through acid hydrolysis of isolated intramuscular connective tissue. There was a significant difference between 7 dpm and 21 dpm soluble collagen percentages when the non-normally distributed data was transformed using the Box-Cox transformation and a paired parametric test was used (Table 3.23). There was no significant difference between the means of 7 dpm and 21 dpm soluble collagen percentages (P = 0.44) when the significance was tested using a non-parametric test.

Table 3.23: Paired t-test for collagen solubility at 7 dpm and 21 dpm with transformed data

	n	Mean % soluble collagen (standard error)	Normality prior to transformation (p-value)	Normality after transformation (p-value)	T-test p-value
7 dpm soluble collagen % of total collagen from pyridinoline assay	49	20.10 (1.38)	0.08	0.94	<0.01*
Aged soluble collagen % of total collagen from pyridinoline assay	49	21.19 (1.05)	0.03*	0.69	

*= Reject null hypothesis

Table 3.24: Wilcoxon test of collagen solubility at 7 dpm and 21 dpm

	n	Mean % soluble collagen (standard error)	P-value
7 dpm soluble collagen % of total collagen from pyridinoline assay	49	20.10 (1.38)	0.44
Aged soluble collagen % of total collagen from pyridinoline assay	49	21.19 (1.05)	

3.2. Study 2A. 2017 Lacombe cattle

3.2.1. Lacombe steroidal treatment

Descriptive statistics for the data from the 2017 cohort of the Lacombe cattle are shown in Table 3.25. There was a broad range of cooking time, fat percentage of the LT, temperature at 72 hours post mortem, yield and quality grades, shear force, and measures of collagen solubility, total collagen, and mature collagen crosslinks. The ranges of moisture content, protein content,

temperature and pH at 45 minutes post mortem, pH at 72 hours post-mortem, and muscle colour were narrow, with low coefficients of variation. The variation of collagen traits is suitable for establishing genetic relationships with these traits.

Table 3.25: Descriptive statistics for Lacombe 2017 animal, carcass and meat quality data

	n	Minimum	Maximum	Mean	Coefficient of Variance
Age at slaughter (days)	88	486	754	624.43	0.12
Cooking loss %	88	14.64	31.77	21.36	0.17
Cooking time (sec/g)	88	1.99	7.1	4.12	0.26
Moisture %	88	68.42	74.28	72.51	0.02
Fat %	88	1.89	9.69	4.01	0.37
Protein %	88	20.87	23.84	22.19	0.02
Temp 45 min post-mortem	88	33.8	40.2	36.65	0.04
Temp 72 hrs post-mortem	86	0.1	2.5	1.17	0.45
pH 45 min post-mortem	88	6.16	6.94	6.66	0.02
pH 72 hrs post-mortem	84	5.35	5.74	5.53	0.01
YG	88	2	5	2.64	0.28
QG	88	1	3	2.22	0.20
Shear (kg)	88	3.25	11.03	5.52	0.24
LT L*	86	32.33	44.44	38.56	0.06
LT a*	86	16.52	25.19	21.19	0.08
LT b*	86	11.42	18.24	14.61	0.09
LT Chroma	86	20.42	30.76	25.75	0.08
LT Hue	86	31.11	38.07	34.62	0.04
Total collagen from tryptic digesta mg/g raw meat	88	1.18	5.27	2.41	0.36
Total collagen from pyridinoline mg/g raw meat	88	2.48	5.78	4.22	0.18
7 dpm percent soluble from pyridinoline total	88	1.66	24.11	9.55	0.43
21 dpm percent soluble from pyridinoline total	88	1.57	38.88	7.99	0.65
Ehrlich chromogen mol/mol collagen	88	0.28	0.97	0.54	0.32
Pyridinoline mol/mol collagen	88	0.09	0.26	0.16	0.21

There were no meaningful differences between steroid treatments for cooking loss and cooking time of the LT, nor was there a difference between treatments for meat composition as moisture, fat, and protein percentages were similar for all treatment groups (Table 3.26). Steroid treatments were associated with increased LT temperatures at 45 minutes and 72 hours post

mortem, with higher temperatures at 45 minutes in samples from PE1T steers than that of the control or PE1 steer LT (Table 3.26). Steers that were treated with either 1 or 2 backgrounding implants had a significantly higher LT temperature than the control group steers at 72 hours, with the highest mean temperature being found in the PE2 steers (Table 3.26). While the use of steroids was not associated with a significant difference in pH measurements at 45 minutes post-mortem ($P > 0.05$), at 72 hours post-mortem LT from steers that received multiple implants had a higher pH, with the highest mean pH being seen in samples from PE2 steers (Table 3.26). Yield grade did not differ between steroid treatment, although quality grade did decrease as the number of steroids increased, with Canada Prime being most associated with the control group that received no implants (Table 3.26). Shear force was highest in the PE2T steers, and lowest in the LT from TA steers, with no meaningful difference between the other treatment groups (Table 3.26).

Table 3.26: Differences in least squares means with standard error (SE) for LT meat quality characteristics due to steroidal implants according to Tukey's HSD

	None (n=16)	PE1 (n=16)	PE2 (n=16)	PE2T (n=16)	PE1T (n=16)	TA (n=8)	p-value
Cooking loss %	22.26 (0.94)	21.30 (0.94)	22.28 (0.94)	21.08 (0.94)	20.00 (0.94)	21.12 (1.33)	0.54
Cooking time sec/g	4.01 (0.27)	4.03 (0.27)	4.44 (0.27)	4.11 (0.27)	4.18 (0.27)	3.84 (0.38)	0.82
Moisture %	72.66 (0.29)	72.73 (0.29)	72.39 (0.29)	72.23 (0.29)	72.83 (0.29)	71.86 (0.41)	0.34
LT % fat	3.93 (0.37)	3.56 (0.37)	4.03 (0.37)	4.43 (0.37)	3.74 (0.37)	4.80 (0.53)	0.36
Protein %	22.04 (0.13)	22.37 (0.13)	22.16 (0.13)	22.16 (0.13)	22.21 (0.13)	22.23 (0.18)	0.61
Temp 45 minutes post-mortem	36.25^{b} (0.34)	35.93^{b} (0.34)	36.94^{ab} (0.34)	36.35^{ab} (0.34)	37.64^{a} (0.34)	36.88^{ab} (0.48)	<0.01*
Temp 72 hours post-mortem[1]	0.41^{a} (0.13)	1.41^{bc} (0.13)	1.60^{c} (0.13)	1.46^{bc} (0.13)	1.01^{b} (0.13)	1.00^{ab} (0.22) (n=6)[1]	<0.01*
pH 45 minutes post-mortem	6.73 (0.04)	6.73 (0.04)	6.57 (0.04)	6.61 (0.04)	6.68 (0.04)	6.65 (0.06)	0.03*
pH 72 hours post-mortem[2]	5.50^{bc} (0.02)	5.47^{c} (0.02) (n=12)[2]	5.59^{a} (0.02)	5.56^{ab} (0.02)	5.52^{abc} (0.02)	5.49^{ab} (0.03)	<0.01*
Yield grade	2.81 (0.18)	3.00 (0.18)	2.56 (0.18)	2.44 (0.18)	2.50 (0.18)	2.38 (0.26)	0.16
Quality grade	2.00^{a} (0.11)	2.06^{ab} (0.11)	2.50^{b} (0.11)	2.25^{ab} (0.11)	2.31^{ab} (0.11)	2.13^{ab} (0.16)	0.01*
Shear	5.38^{ab} (0.34)	5.04^{ab} (0.34)	5.93^{ab} (0.34)	6.33^{a} (0.34)	5.47^{ab} (0.34)	4.45^{b} (0.47)	0.01*

[a,b,c] Means with different superscripts in the same row are different at $P < 0.05$.
* indicates a significant p-value of <0.05.
None: No implant, PE1: 1 backgrounding implant, PE2: 2 backgrounding implants, PE1T: 1 backgrounding implant, 1 terminal implant, PE2T: 2 backgrounding implants, 1 terminal implant
TA: 1 terminal implant
[1]n=6: Only 6 animals were measured
[2]n=12: Only 12 animals were measured

There was no significant difference in LT colour measures between treatments, with the mean values of L*, a*, b*, chroma and hue similar across all treatments (Table 3.27).

Based on the results of Tukey's HSD test, total collagen when determined from tryptic digesta for Ehrlich chromogen quantification and isolated IMCT from the pyridinoline assay did

not differ between steroidal implant groups (Table 3.28). Collagen solubility did not significantly differ between steroidal treatment groups when soluble collagen for both aged and 7 dpm samples was expressed as a percentage of total collagen, and when total collagen was determined via hydroxyproline assay from isolated intramuscular connective tissue (Table 3.28). Also, the use of steroids was not associated with changes in mature collagen crosslink content ($P > 0.05$), with no significant difference of mean Ehrlich chromogen or mean pyridinoline content seen across treatment groups.

Table 3.27: Differences in least squares means with standard error (SE) for LT colour values due to steroidal implants according to Tukey's HSD

	None (n=16)	PE1 (n=16)	PE2 (n=16)	PE2T (n=16)	PE1T (n=16)	TA (n=6)	p-value
LT L*	37.82 (0.63)	38.85 (0.63)	38.86 (0.63)	39.96 (0.63)	37.82 (0.63)	36.71 (1.03)	0.05*
LT a*	21.73 (0.42)	20.47 (0.42)	20.82 (0.42)	21.42 (0.42)	21.41 (0.42)	21.48 (0.69)	0.31
LT b*	14.77 (0.32)	14.37 (0.32)	14.14 (0.32)	15.22 (0.32)	14.54 (0.32)	14.67 (0.53)	0.26
LT Chroma	26.29 (0.51)	25.02 (0.51)	25.18 (0.51)	26.29 (0.51)	25.89 (0.51)	26.03 (0.83)	0.33
LT Hue	34.31 (0.37)	35.08 (0.37)	34.22 (0.37)	35.41 (0.37)	34.15 (0.37)	34.30 (0.60)	0.07

None: No implant
PE1: 1 backgrounding implant
PE2: 2 backgrounding implants
PE1T: 1 backgrounding implant, 1 terminal implant
PE2T: 2 backgrounding implants, 1 terminal implant
TA: 1 terminal implant

Table 3.28: Differences in least squares means with standard error (SE) for Lacombe 2017 LT collagen characteristics due to steroidal implants according to Tukey's HSD

	None (n=16)	PE1 (n=16)	PE2 (n=16)	PE2T (n=16)	PE1T (n=16)	TA (n=8)	p-value
Total collagen tryptic digesta mg/g raw meat	2.19 (0.22)	2.50 (0.22)	2.61 (0.22)	2.28 (0.22)	2.57 (0.22)	2.19 (0.30)	0.80
Total collagen from pyridinoline	4.00 (0.19)	4.21 (0.19)	4.17 (0.19)	4.52 (0.19)	4.01 (0.19)	4.56 (0.27)	0.24
7 dpm percent soluble from pyridinoline	9.83 (1.03)	8.51 (1.03)	10.65 (1.03)	9.11 (1.03)	9.17 (1.03)	10.51 (1.46)	0.72
21 dpm percent soluble from pyridinoline	7.02 (1.31)	5.93 (1.31)	8.95 (1.31)	9.23 (1.31)	8.48 (1.31)	8.65 (1.85)	0.46
Ehrlich chromogen mol/mol collagen	0.56 (0.04)	0.51 (0.04)	0.49 (0.04)	0.61 (0.04)	0.50 (0.04)	0.62 (0.06)	0.25
Pyridinoline mol/mol collagen	0.16 (0.01)	0.15 (0.01)	0.16 (0.01)	0.16 (0.01)	0.17 (0.01)	0.15 (0.01)	0.42

None: No implant
PE1: 1 backgrounding implant
PE2: 2 backgrounding implants
PE1T: 1 backgrounding implant, 1 terminal implant
PE2T: 2 backgrounding implants, 1 terminal implant
TA: 1 terminal implant

3.2.2. Correlations between collagen and meat quality characteristics (Lacombe 2017 steers)

There were no significant correlations between collagen and meat quality characteristics (Table 3.29).

Table 3.29: Pearson correlation of collagen and meat quality characteristics for Lacombe 2017 samples

	Tryptic digesta total collagen	Pyridinline total collagen	7 dpm % soluble collagen	21 dpm % soluble collagen	Ehrlich's chromogen	Pyridinoline
Age at slaughter	0.10	0.09	-0.05	0.07	-0.06	0.05
Moisture %	-0.06	-0.14	0.01	-0.11	0.02	0.16
Rib fat %	0.05	0.11	-0.02	0.13	-0.02	-0.13
Protein %	-0.07	0.12	0.03	-0.19	0.07	-0.01
LT L*	0.02	0.09	-0.07	0.08	0.04	0.04
LT a*	-0.12	0.02	-0.01	-0.14	0.14	-0.12
LT b*	-0.14	0.13	-0.07	-0.15	0.19	-0.12
LT Chroma	-0.14	0.06	-0.03	-0.15	0.17	-0.13
LT Hue	-0.05	0.18	-0.10	-0.06	0.10	-0.01
Shear	0.06	-0.05	0.07	0.07	-0.04	0.08
Cooking loss %	0.00	-0.04	-0.01	0.04	0.07	-0.02
Cook time (sec/g)	0.05	-0.06	-0.06	0.14	-0.01	0.00
Temp. 45 mins	-0.01	0.03	0.01	0.20	0.03	0.03
Temp. 72 hrs	0.00	0.06	-0.14	0.06	0.03	-0.10
pH 45 mins	-0.13	-0.05	-0.02	-0.05	0.05	-0.02
pH 72 hrs	0.00	0.06	-0.14	0.06	0.03	-0.10
Yield grade	0.15	-0.16	-0.11	0.03	-0.14	0.03
Quality grade	-0.18	-0.18	-0.02	0.17	0.08	0.12

* Correlations with an asterisk denote a significant correlation with a P-value < 0.05

3.2.3. Multiple linear regression for shear force

The multiple regression stepwise selection determined that the formula for predicting shear force (kg) for the Lacombe 2017 samples included age at slaughter (P = 0.0051), LT chroma (P = 0.0152), and cooking loss (P = 0.0066). This regression equation explained 18.44% of the variation in shear force.

The regression equation for shear force as determined by the stepwise regression analysis is: Shear force = 4.417402 + 0.005005*(age at slaughter) - 0.161740*(LT chroma) + 0.101424*(cooking loss %).

3.2.4 Collagen solubility over time

There was a significant effect of aging on the heat solubility of collagen (P = 0.029). The use steroidal implants did not have a significant effect on collagen solubility when solubility was expressed as a percentage of total collagen as determined through acid hydrolysis of the intramuscular connective tissue (P > 0.05). There was also no significant interaction between the use of steroids and the aging treatment (P > 0.05).

Table 3.30: Split plot ANOVA output for steroid and aging effects on collagen solubility (with standard errors) for the Lacombe 2017 cohort

		n	Mean % collagen solubility	P-value	Interaction p-value
Aging	Collagen solubility of 7 dpm samples	88	9.55 (0.50)	0.03	0.30
	Collagen solubility of 21 dpm samples	88	7.99 (0.50)		
Steroid	No steroid	32	8.42 (0.83)	0.21	
	1st implant	32	7.22 (0.83)		
	1st and 2nd implant	32	9.80 (0.83)		
	1st and 2nd and terminal implant	32	9.17 (0.83)		
	1st and terminal implant	32	8.83 (0.83)		
	Terminal implant only	16	9.58 (1.18)		

3.3 Study 2B. 2018 Lacombe cattle

3.3.1. Lacombe steroidal treatment

Descriptive statistics for the data from the 2018 cohort of the Lacombe cattle are shown in Table 3.31. There was a broad range of cooking loss and cooking time, fat percent of the carcass, temperature at 72 hours post mortem, yield and quality grades, shear force, and measures of total collagen, collagen solubility, and mature crosslinks of collagen. The ranges of animal age at slaughter, moisture content, protein content, carcass temperature at 45 minutes post-mortem, pH at 45 minutes and 72 hours post-mortem, and muscle colour were narrow, with low coefficients of variation, indicating low variation of these traits in this group of animals. The

moderate to broad range of collagen characteristics make these traits suitable for establishing a genetic relationship.

Table 3.31: Descriptive statistics for Lacombe 2018 animal, carcass and meat quality data

	n	Minimum	Maximum	Mean	Coefficients of Variation
Age at slaughter	61	418	555	486.61	0.07
Cooking loss %	61	15.07	33.95	21.72	0.17
Cooking time, sec/g	61	2.67	7.2	4.33	0.24
Moisture %	61	67.88	74.95	71.89	0.02
Fat %	61	1.44	10.04	4.90	0.39
Protein %	61	20.79	23	22.05	0.02
Temp 45 min post-mortem	61	33.2	39.5	36.19	0.04
Temp 72 hrs post-mortem	59	0.1	2.9	1.15	0.59
pH 45 min post-mortem	61	6.28	7.08	6.69	0.02
pH 72 hrs	61	5.41	5.7	5.54	0.01
YG	61	1	5	2.43	0.32
QG	61	1	3	2.13	0.20
Shear	61	3.65	10.1	6.11	0.26
LT L*	59	33.88	41.64	38.00	0.06
LT a*	59	16.53	24.73	21.02	0.08
LT b*	59	11.7	17.43	14.45	0.08
LT Chroma	59	20.41	29.41	25.52	0.07
LT Hue	59	31.32	37.91	34.53	0.04
Total collagen from tryptic digesta mg/g raw meat	61	1.08	5.07	2.20	0.35
Total collagen from pyridinoline	61	2.60	6.09	4.15	0.18
7 dpm percent soluble from pyridinoline total	61	3.34	26.24	12.16	0.43
21 dpm percent soluble from pyridinoline total	61	0.57	41.78	10.06	0.73
Ehrlich chromogen mol/mol collagen	61	0.3	0.99	0.56	0.28
Pyridinoline mol/mol collagen	61	0.11	0.23	0.15	0.17

Cooking loss and cook time did not differ significantly between the control muscles and the muscles from steers that received the terminal implant in the 2018 cohort of the Lacombe cattle, nor was there a significant difference between the treated and untreated muscles for mean percentage of LT moisture, fat, or protein (Table 3.32). The terminal steroid was not associated with a mean difference in LT temperatures or pH measurements at 45 minutes post mortem (P >

0.05), however temperature and pH measurements were both significantly higher in the treated samples at 72 hours post-mortem. Yield grade decreased in the implanted group, although quality grade and shear force did not differ (Table 3.32).

Table 3.32: Differences in least squares means with standard error (SE) for Lacombe 2018 LT meat quality characteristics due to steroidal implants according to Tukey's HSD

	None (n = 31)	Terminal (n = 30)	p-value
Cooking loss %	21.60 (0.73)	21.85 (0.75)	0.81
Cooktime sec/g	4.18 (0.19)	4.48 (0.19)	0.28
Moisture %	72.09 (0.28)	71.70 (0.29)	0.34
LT % fat	4.75 (0.34)	5.05 (0.35)	0.53
Protein %	22.09 (0.09)	22.00 (0.09)	0.49
Temp 45 minutes post-mortem	36.37 (0.26)	36.00 (0.26)	0.32
Temp 72 hours post-mortem	0.91[a] (0.12)	1.42[b] (0.13) (n=28)[1]	<0.01*
pH 45 minutes post-mortem	6.70 (0.03)	6.68 (0.03)	0.59
pH 72 hours post-mortem	5.52[a] (0.01)	5.56[b] (0.01)	0.01*
Yield grade	2.71[a] (0.14)	2.13[b] (0.14)	<0.01*
Quality grade	2.03 (0.08)	2.23 (0.08)	0.07
Shear	6.07 (0.29)	6.15 (0.29)	0.85

[a,b] Means with different superscripts in the same row are different at $P < 0.05$.
* indicates a significant p-value of <0.05.
[1]n=28: Temperature at 72 hours post-mortem was measured in only 28 samples from the terminal group

Values of L* in the Lacombe 2018 cohort were significantly higher in cattle that were not treated with a terminal implant ($P = 0.0178$). None of the other measured values for *LT* colour, including a*, b*, chroma, or hue were significantly different between the treatment group that received a terminal steroid and the control group in the Lacombe 2018 samples (Table 3.33).

Total collagen as determined from tryptic digesta and from isolated IMCT as part of the pyridinoline assay were similar between treatment groups (Table 3.34). Collagen solubility of both 7 dpm and 21 dpm samples did not differ significantly between the treated and untreated groups (Table 3.34). Mean values of Ehrlich chromogen and pyridinoline concentrations did not significantly differ between treated and untreated samples ($P > 0.05$).

Table 3.33: Differences in least squares means with standard error (SE) for LT colour due to steroidal implants according to Tukey's HSD

	None (n = 31)	Terminal (n = 28)	p-value
LT L*	38.65[a] (0.40)	37.28[b] (0.42)	0.02*
LT a*	20.75 (0.29)	21.31 (0.31)	0.19
LT b*	14.29 (0.22)	14.62 (0.23)	0.30
LT Chroma	25.22 (0.34)	25.86 (0.36)	0.20
LT Hue	34.63 (0.27)	34.43 (0.28)	0.61

[a,b] Means with different superscripts in the same row are different at $P < 0.05$.
* indicates a significant p-value of <0.05.

Table 3.34: Differences in least squares means with standard error (SE) for LT collagen characteristics due to steroidal implants according to Tukey's HSD

	None (n=31)	Terminal (n=30)	p-value
Total collagen tryptic digesta mg/g raw meat	2.16 (0.14)	2.25 (0.15)	0.65
Total collagen from pyridinoline	4.05 (0.14)	4.26 (0.14)	0.30
7 dpm percent soluble from pyridinoline	12.62 (0.95)	11.68 (0.97)	0.50
21 dpm percent soluble from pyridinoline	9.61 (1.33)	10.52 (1.36)	0.63
Ehrlich chromogen mol/mol collagen	0.57 (0.03)	0.56 (0.03)	0.86
Pyridinoline mol/mol collagen	0.16 (<0.01)	0.14 (<0.01)	0.06

3.3.2. Correlations between collagen and meat quality characteristics of Lacombe 2018 steers

There were no significant correlations between any collagen characteristics and moisture, rib fat percent, LT a*, b*, chroma or hue values, shear force, cooking loss, cook time, temperature at 45 minutes post mortem, pH at 45 minutes post mortem, or pH at 72 hours post mortem (Table 3.30). LT L* values correlated significantly and negatively with total collagen when measured from hydrolyzed IMCT ($P=0.02$, $r = -0.29$) but were not significantly correlated with any other collagen characteristics. The percentage of soluble collagen in aged samples significantly decreased as age at slaughter increased ($P = 0.01$, $r = -0.33$), and the percentage of soluble collagen in aged samples significantly increased as protein % decreased ($P = 0.01$, $r =$

-0.32). An increase in temperature at 72 hours post mortem was significantly correlated with an increase in the percentage of soluble collagen in 7 dpm and 21 dpm samples (P=0.04, r = 0.27 and 0.02, r = 0.31 respectively). Yield grade was significantly correlated with 7 dpm collagen solubility expressed as a percentage of total collagen (P=0.04, -0.26), with collagen solubility being higher in Canada Prime cuts than AA, and the percent of aged soluble collagen increased as quality grade increased (P = 0.04, r = 0.26).

Table 3.35: Pearson correlation coefficients between collagen and meat quality characteristics of Lacombe 2018 samples

	Ehrlich's chromogen total collagen	Pyridinoline total collagen	7 dpm % sol.	21 dpm % sol.	Ehrlich's chromogen	Pyridinoline
Age at slaughter	0.00	0.15	-0.24	-0.33*	0.11	0.22
Moisture %	-0.05	-0.97	-0.02	-0.17	0.06	0.05
Rib fat %	-0.02	0.04	0.01	0.20	0.01	-0.06
Protein %	-0.01	-0.50	-0.09	-0.32*	0.07	0.00
LT L*	-0.06	-0.29*	-0.10	0.02	-0.06	0.13
LT a*	0.01	-0.05	0.14	-0.13	-0.04	-0.08
LT b*	0.02	-0.14	0.05	-0.04	-0.10	-0.05
LT Chroma	0.01	-0.09	0.12	-0.11	-0.06	-0.08
LT Hue	0.01	-0.15	-0.15	0.11	-0.09	0.06
Shear	-0.06	0.13	0.14	0.05	0.02	-0.15
Cooking loss %	0.12	-0.08	0.02	-0.16	-0.14	<0.01
Cook time sec/g	0.16	0.10	-0.06	0.02	-0.05	-0.13
Temp. 45 mins	-0.04	-0.06	0.07	-0.07	-0.05	-0.21
Temp. 72 hrs	-0.01	0.05	0.27*	0.31*	-0.02	0.02
pH 45 mins	-0.01	0.05	-0.11	0.14	-0.02	0.02
pH 72 hrs	0.16	0.10	0.27	0.31	-0.17	-0.24
Yield grade	-0.01	-0.11	-0.26*	-0.17	-0.04	0.24
Quality grade	-0.08	-0.17	<0.01	0.26*	-0.02	-0.10

* Correlations with an asterisk denote a significant correlation with a P-value < 0.05

3.3.3. Multiple linear regression for shear force

The formula for predicting shear force (kg) for the Lacombe 2018 samples was determined using a multiple regression stepwise selection. The factors included in the determination of shear force were age at slaughter (P = 0.00045), *LT* b* (P = 0.01827), and the percent of cooking loss (P = 0.00034). This regression equation explained 35.13% of the variation in shear force.

The regression equation for shear force as determined by the stepwise regression analysis is:

Shear force = 15.73488 – 0.01717*(age at slaughter) – 0.32087*(*LT* b*) + 0.15621*(cooking loss %).

3.3.4. Collagen solubility over time

There was no significant effect of aging on the heat solubility of collagen (P > 0.05). The use of a terminal implant did not have a significant effect on collagen solubility when solubility was expressed as a percentage of total collagen as determined through acid hydrolysis of the intramuscular connective tissue. There was also no significant interaction between the use of steroids and the aging treatment.

Table 3.36: Means (with standard errors) for ANOVA testing the effects of steroid and aging on collagen solubility, Lacombe 2018

	Mean % collagen solubility	P-value	Interaction p-value
Collagen solubility of 7 dpm samples (n = 61)	12.16 (0.83)	0.07	0.42
Collagen solubility of 21 dpm samples (n = 61)	10.06 (0.83)		
Collagen solubility of untreated beef (n = 62)	11.11 (0.83)	0.99	
Collagen solubility of treated beef (n = 60)	11.10 (0.84)		

3.4. Study 3: Genetic effect on collagen characteristics

3.4.1. Heritability and correlations

The variance components and heritability estimates are presented in Table 3.37. Collagen solubility at 7 dpm and the content of the mature crosslinks Ehrlich's chromogen and

pyridinoline were lowly heritable. Total collagen showed moderate heritability ($h^2 = 0.3822$) despite the heritability of total collagen varying widely. Collagen solubility after aging had the highest degree of heritability of these five traits.

Table 3.37: Estimation of mean heritability (h^2) with standard error (SE) for collagen characteristics

	Additive variance (σ_a^2) (SE)	Residual variance (σ_e^2) (SE)	Heritability (h^2) (SE)
Unaged collagen solubility	24.79 (23.79)	152.87 (23.54)	0.1364 (0.1236)
Aged collagen solubility	90.60 (37.92)	61.61 (30.26)	0.5852 (0.2137)
Total collagen	1.489 (0.8881)	2.318 (0.7405)	0.3822 (0.2067)
Ehrlich's chromogen	0.0079 (0.0075)	0.0237 (0.0064)	0.2399 (0.2036)
Pyridinoline	0.0003 (0.0002)	0.0008 (0.0002)	0.2437 (0.1823)

The genotypic and phenotypic correlations were also estimated between all five traits. Collagen solubility prior to aging was highly genetically correlated with total collagen, but this correlation was not present phenotypically (Table 3.38). Unaged collagen solubility was also highly genetically correlated with pyridinoline content (50.33%), though this relationship was not seen phenotypically. Total collagen and pyridinoline were highly negatively correlated both genetically and phenotypically (Table 3.38). All other correlations between traits were negligible.

Table 3.38: Genotypic (above the diagonal) and phenotypic (beneath the diagonal) correlation of collagen characteristics

	Collagen solubility prior to aging	Collagen solubility after aging	Total collagen	Ehrlich's chromogen	Pyridinoline
Collagen solubility prior to aging		-0.1933 (0.6024)	0.7698 (0.3168)	0.0680 (0.7265)	0.5033 (0.5852)
Collagen solubility after aging	0.0456 (0.0780)		0.1466 (0.4774)	-0.4457 (0.6253)	0.1677 (0.6686)
Total collagen	0.1878 (0.0681)	-0.2420 (0.0703)		0.4351 (0.6405)	-0.9042 (0.1835)

Ehrlich's chromogen	0.2476 (0.2173)	-0.0939 (0.1808)	0.1168 (0.2122)		-0.2582 (0.6321)
Pyridinoline	0.2297 (0.2167)	0.1040 (0.1836)	-0.7144 (0.0876)	-0.0680 (0.1042)	

*SE in brackets

3.4.2. Genome-wide association study

A total of 5 SNP windows that explained more than 1% of the additive genetic variance were found for collagen solubility prior to aging, and 8 SNP windows were detected for the solubility of collagen after aging (Table 3.39). The SNP windows on chromosome 19 (50,787,009-51,325,894) and chromosome 16 (71,463,111-72,311,987) explained the greatest amount of additive genetic variance (2.32% and 4.19%) for collagen solubility before and after aging respectively. There were 5 SNP windows that accounted for more than 1% of the variance in total collagen, with the SNP window on chromosome 8 (52,189,823-52,932,839) explaining 2.52% of the variance. Finally, 4 SNP windows explained more than 1% of the additive variance for Ehrlich's chromogen, and a total of 10 SNP windows were identified that accounted for more than 1% of the additive variance of pyridinoline. The SNP window on chromosome 20 (47,857,745-48,758,899) explained 2.97% of the variance for Ehrlich's chromogen, and for pyridinoline the SNP window on chromosome 23 (35,579,146-36,075,197) explained 1.74%. In total these SNP windows explained 7.84% and 13.57% of the additive genetic variance of collagen solubility before and after aging, respectively, 7.72% of total collagen, and 7.71% and 15.02% of Ehrlich's chromogen and pyridinoline respectively. Manhattan plots for the five collagen characteristics are presented in Figures 3.1-3.5.

While significant SNP windows could be found on the same chromosome for different measured traits as shown in Table 3.39 (such as chromosome 5 for collagen solubility before and after aging and pyridinoline, or chromosome 1 for total collagen and pyridinoline), there was no

overlap between any of the significant SNP windows for any of the collagen traits that were measured in this study. There was also no overlap between the SNP windows that were found to be significant for this study, and those that were found to be significant for the 137 steers in a previous study.

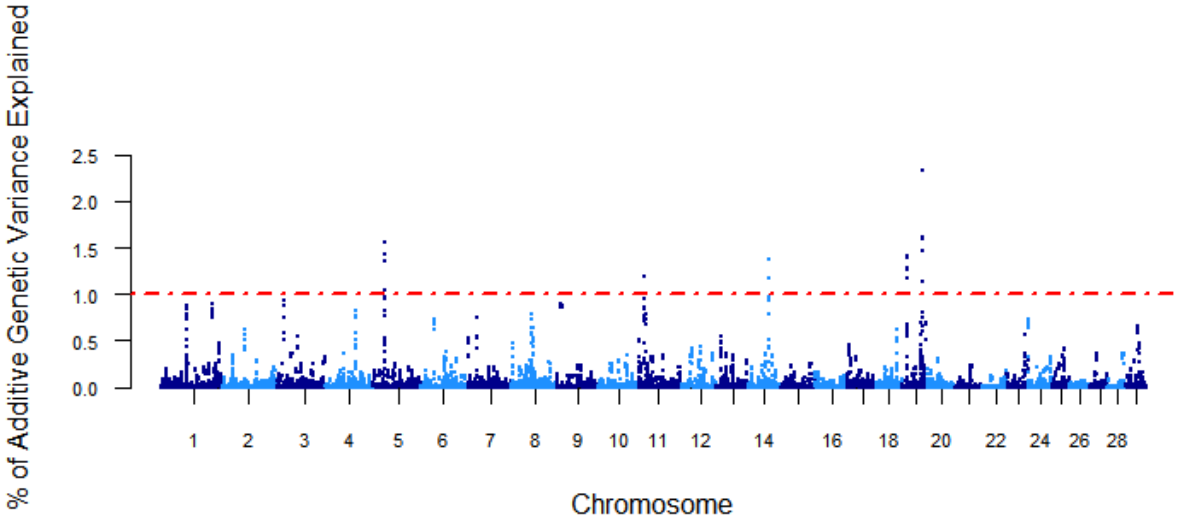

Figure 3.1: Manhattan plot depicting the percent variance explained by SNP windows in collagen solubility in *longissimus* muscle prior to aging, dotted line indicates 1% threshold for total additive genetic variance explained by SNP window.

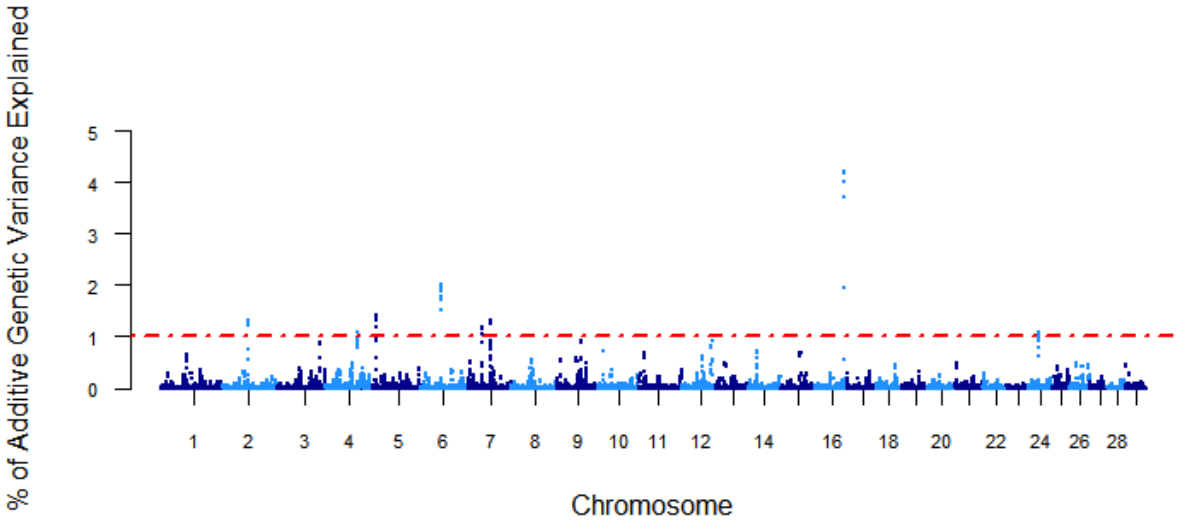

Figure 3.2: Manhattan plot depicting the percent variance explained by SNP windows in collagen solubility in *longissimus* muscle after aging, dotted line indicates 1% threshold for total additive genetic variance explained by SNP window.

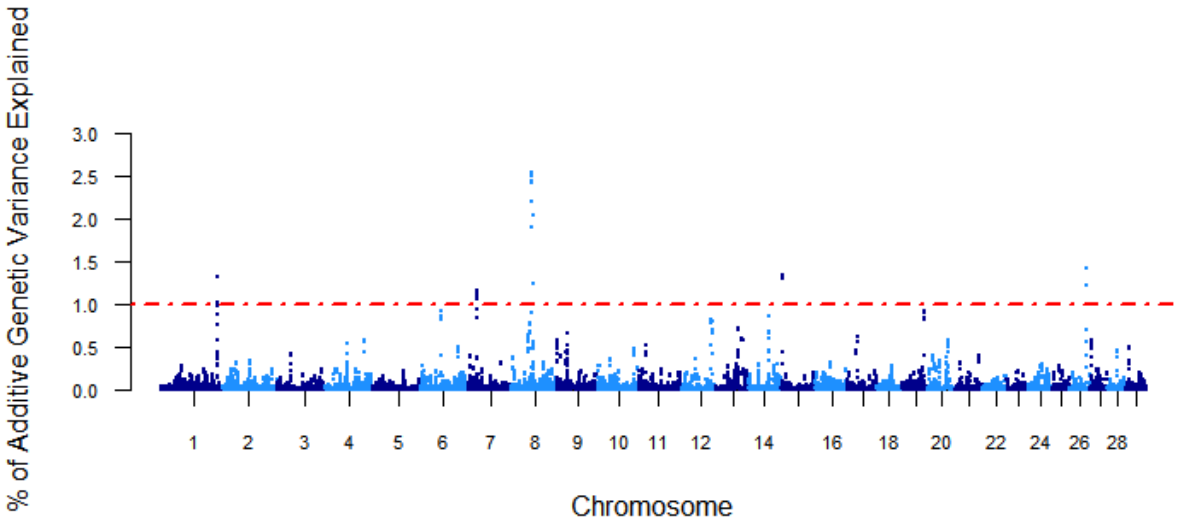

Figure 3.3: Manhattan plot depicting the percent variance explained by SNP windows in total collagen in the *longissimus* muscle, dotted line indicates 1% threshold for total additive genetic variance explained by SNP window.

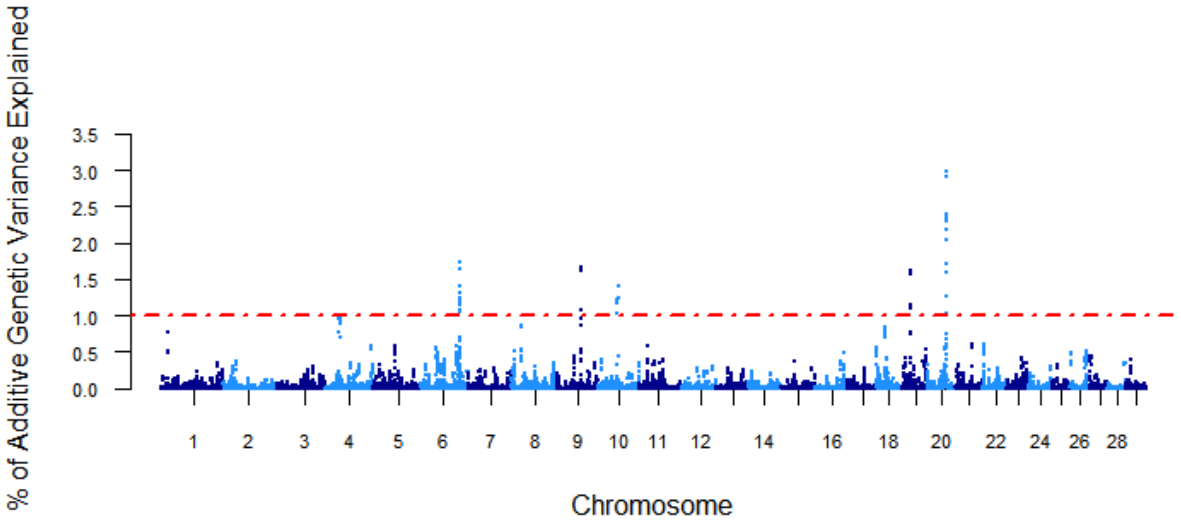

Figure 3.4: Manhattan plot depicting the percent variance explained by SNP windows in Ehrlich's chromogen crosslink in the *longissimus* muscle, dotted line indicates 1% threshold for total additive genetic variance explained by SNP window.

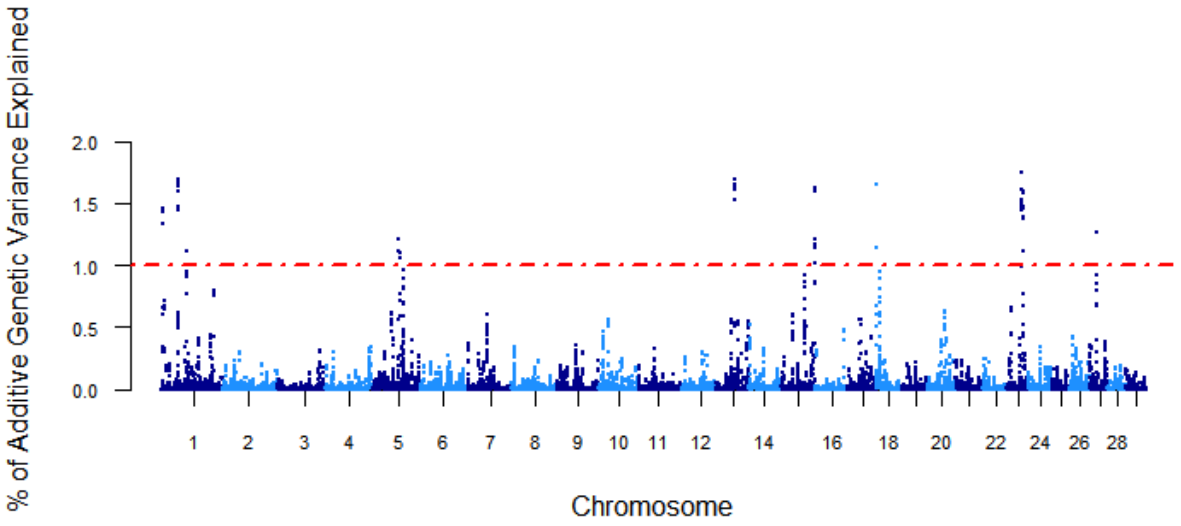

Figure 3.5: Manhattan plot depicting the percent variance explained by SNP windows in pyridinoline crosslink content of the *longissimus* muscle, dotted line indicates 1% threshold for total additive genetic variance explained by SNP window.

3.4.3. Functional analysis

RefSeq genes searched through the Biomart tool in Ensembl's genetic database (release version 107, ARS-UCD1.2) were used for identification. Some of the genes that were identified by Ensembl's database are of unknown function but are known to be protein coding and so were still included in these results. Within the SNP windows that explained more than 1% of the additive genetic variance (within 500 Kb and 500 Kb downstream), 82 protein coding genes on 4 chromosomes were identified for collagen solubility prior to aging (Table 3.39). For the collagen solubility of aged samples, 28 genes on 6 different chromosomes were found, and 29 genes on 5 different chromosomes were found for total collagen (Table 3.39). There were 21 genes on 2

chromosomes identified for Ehrlich's chromogen, and 73 genes on 7 chromosomes were found pyridinoline (Table 3.39). As there were no overlapping SNP windows for any of the selected traits there were also no common genes.

Table 3.39: Protein coding genes within SNP windows significant to collagen characteristics

Trait	Chr[1]	SNP Window		Var[2]	Genes
		Start BP	End BP		
Unaged collagen solubility	5	29760798	30247421	1.55462	CERS5, COX14, GPD1, SMARCD1, ASIC1, RACGAP1, AQP5, AQP2, FAIM2, BCDIN3D, NCKAP5L, TMBIM6, FMNL3, PRPF40B, FAM186B
	11	15901243	16384871	1.18543	LTBP1, RASGRP3, FAM98A
	14	51532633	52169591	1.37621	CSMD3
	19	14094861	14788290	1.40579	HEATR6, CCL3, WFDC18, CCL4, CCL14, CCL16, CCL5, HEATR9, TAF15, MMP28, C19H17orf50, GAS2L2, RASL10B, AP2B1, PEX12, SLFN14
	19	50787009	51325894	2.32061	FASN, DUS1L, GPS1, RFNG, DCXR, RAC3, DCXR, LRRC45, CENPX, ASPSCR1, NOTUM, MYADML2, PYCR1, MAFG, SIRT7, PCYT2, NPB, ANAPC11, ALYREF, ARHGDIA, P4HB, PPP1R27, MCRIP1, GCGR, SLC25A10, MRPL12, HGS, ARL16, CCDC137, OXLD1, PDE6G, TSPAN10, NPLOC4, FAAP100, FSCN2, ACTG1, BAHCC1
Aged collagen solubility	2	64040881	64731387	1.31426	NCKAP5
	4	80423235	81185186	1.0753	CDK13, MPLKIP, SUGCT
	5	6477926	6923652	1.41083	E2F7
	6	49974410	50672369	2.02268	PCDH7
	7	36136185	37093380	1.16568	
	7	59255672	59993171	1.29923	HTR4, FBXO38, SPINK5, SPINK6, SPINK7, SPINK9

	16	71463111	72311987	4.18998	DTL, INTS7, LPGAT1, NEK2, KCNH1, RD3, RCOR3, SLC30A1, TRAF5
	24	27472545	27930734	1.08777	
Total Collagen	1	143134443	143725156	1.32029	CRYAA, CBS, HSF2BP, PKNOX1, U2AF1
	7	21699684	22273701	1.14235	IRF1, IL13, IL4, IL5, PDLIM4, P4HA2, RAD50, SLC22A4, SLC22A5
	8	52189823	52932839	2.52357	GCNT1, PCSK5, PRUNE2, RFK
	15	2213562	3192179	1.32303	GRIA4
	26	43709270	44437830	1.40895	ABRAXAS2, CHST15, FAM53B, NKX1-2, OAT, LHPP
Ehrlich's chromogen	6	98404504	99019571	1.72147	
	10	50532545	51206364	1.39742	BNIP2, CCNB2, FAM81A, GCNT3, MYO1E, GTF2A2
	19	20472697	21317170	1.62578	ABHD15, ANKRD13B, CORO6, CRYBA1, EFCAB5, GIT1, NUFIP2, NSRP1, SSH2, SLC6A4, TAOK1, TP53I13
	20	47857745	48758899	2.96968	
Pyridinoline	1	8298027	9069741	1.45024	H4C3, H4C3
	1	46242001	46810734	1.69149	HACD2, ADCY5, CCDC14, MYLK, ROPN1, SEC22A
	1	67619208	68440795	1.10717	
	5	65645919	66603000	1.2043	DRAM1, IGF1, GNPTAB, NUP37, PARPBP, PMCH, WASHC3
	13	48156674	49257439	1.68873	BMP2, CRLS1, FERMT1, LRRN4, MCM8
	15	82797506	83309084	1.61988	CBLIF, MS4A1, MS4A14, MS4A2, MS4A3, MS4A5, MRPL16, OR10V1, OOSP2, OSBP, PATL1, STX3, TCN1
	18	2443636	2932707	1.65510	ADAT1, BCAR1, CHST6, CFDP1, CFDP2, GABARAPL2, LDHD, KARS1, TMEM170A, TMEM231, ZFP1, ZNRF1
	23	35579146	36075197	1.73968	HDGFL1
	23	39665286	40480107	1.59357	CAP2, FAM8A1, KIF13A, NUP153, RBM24, STMND1
	27	18847512	19744617	1.26539	FGL1, MTUS1, ASAH1, PCM1, PDGFRL, SLC7A2

1: Chromosome number
2: Additive genetic variance

The individual SNP windows that explained the greatest amount of variance were extended an additional 500 Kb both upstream and downstream to look for further genes that may be in linkage disequilibrium with the identified SNP. 56 genes were identified for collagen solubility prior to aging, 14 genes were found for collagen solubility after aging, 9 genes were significant for total collagen, only 1 protein coding gene was found in the extended SNP window that explained the greatest amount of variance in Ehrlich's chromogen, and 6 genes were identified for pyridinoline (Table 3.40).

Table 3.40: Genes within extended SNP windows explaining the greatest additive variance

Trait	Chr[1]	SNP Window		Genes
		Start BP	End BP	
Unaged collagen solubility	19	50787009	51325894	SECTM1A, SECTM1, CD7, CSNK1D, SLC16A3, CCDC57, FASN, DUS1L, GPS1, RFNG, DCXR, RAC3, DCXR, LRRC45, CENPX, ASPSCR1, NOTUM, MYADML2, PYCR1, MAFG, SIRT7, PCYT2, NPB, ANAPC11, ALYREF, ARHGDIA, P4HB, PPP1R27, MCRIP1, GCGR, SLC25A10, MRPL12, HGS, ARL16, CCDC137, OXLD1, PDE6G, TSPAN10, NPLOC4, FAAP100, FSCN2, ACTG1, BAHCC1, SLC38A10, NDUFAF8, TEPSIN, CEP131, AATK, BAIAP2, CHMP6, RPTOR
Aged collagen solubility	16	71463111	72311987	NENF, PACC1, PPP2R5A, DTL, INTS7, LPGAT1, NEK2, SLC30A1, RD3, TRAF5, RCOR3, KCNH1, HHAT
Total collagen	8	52189823	52932839	PCSK5, RFK, GCNT1, PRUNE2, FOXB2, VPS13A
Ehrlich's chromogen	20	47857745	48758899	CDH10
Pyridinoline	23	35579146	36075197	PRP3, CSH2, PRL, HDGFL1, SOX4

1: Chromosome number

3.4.4. Genetic network analysis

Ingenuity Pathway Analysis software was used to determine the physiological networks in which the genes were involved. The contents of Tables 3.39 and 3.40 were used to inform the software. The gene networks identified as the top physiological functions for the genes in the SNP windows that explained the greatest amount of additive genetic variance were *i*: Auditory disease, neurological disease, organismal injury and abnormalities (Figure 3.6), *ii*: Cancer, cell cycle, organismal injury and abnormalities (Figure 3.7), iii: Cell signalling, molecular transport, nucleic acid metabolism (Figure 3.8), *iv*: Cellular development, hematological system development and function, lymphoid tissue structure and development (Figure 3.8), and *v*: Cell morphology, cellular function and maintenance, cell death and survival (Figure 3.9). Networks iii and iv were connected through the gene *NPS* and are presented as a single merged network. *NPS* was not found in the significant SNP windows in the current study and was instead connected to the networks as determined by the IPA software. The genes found within the significant SNP windows were also input into the IPA software to determine the pathways that were specific to each individual collagen trait of the five that were measured. The top physiological functions associated with the individual traits when those traits were analyzed in isolation involved organismal injury and abnormalities, cell signaling, cellular development and movement, cell death and survival, and cancer, among others. The genes within the SNP windows that were found to be significant for pyridinoline were the only genes that were found to be part of a network connected to collagen forming genes (Figure 3.10).

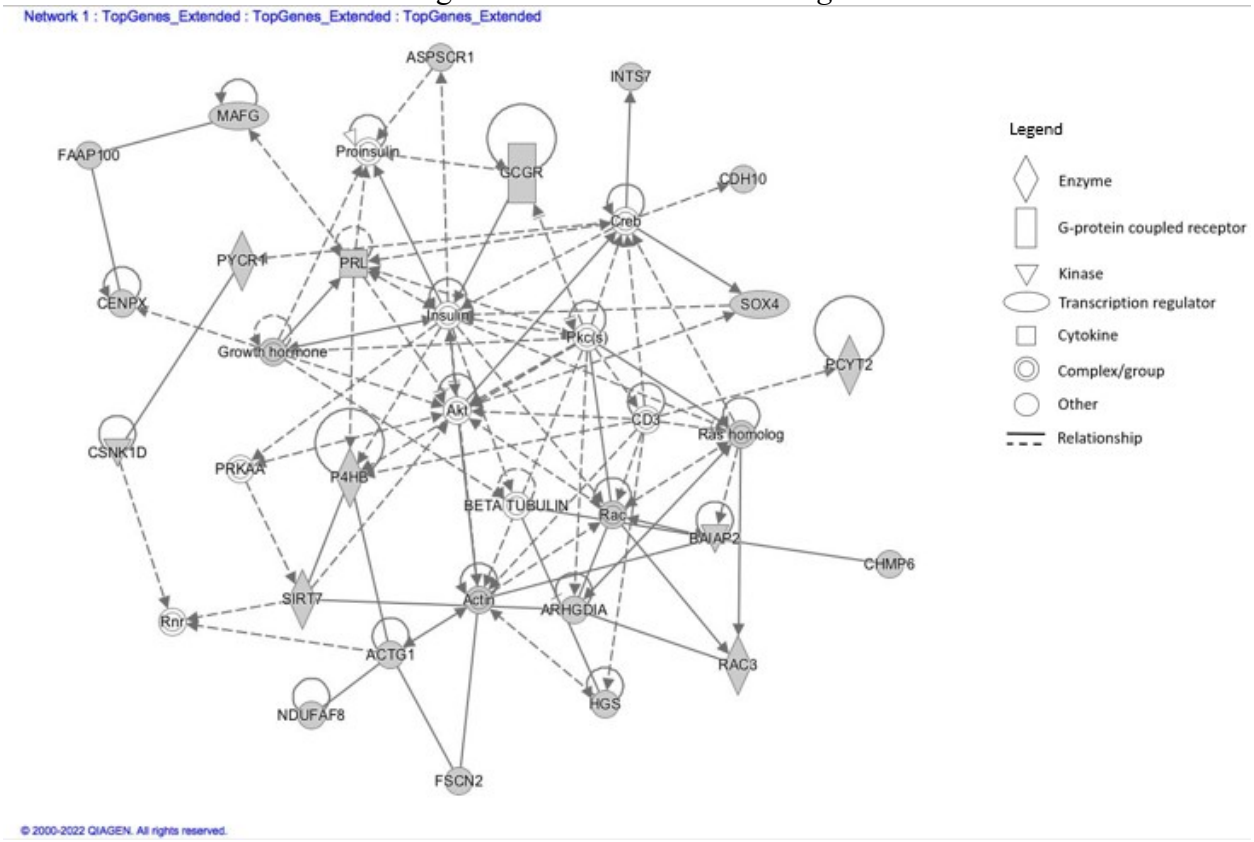

Figure 3.6: Genes identified in proximity to SNP windows that explained >1% variance for collagen characteristics in beef *longissimus* muscle, and their involvement in the auditory disease, neurological disease, and organismal injury and abnormalities pathway.

GWAS for collagen characteristics in beef *longissimus* muscle

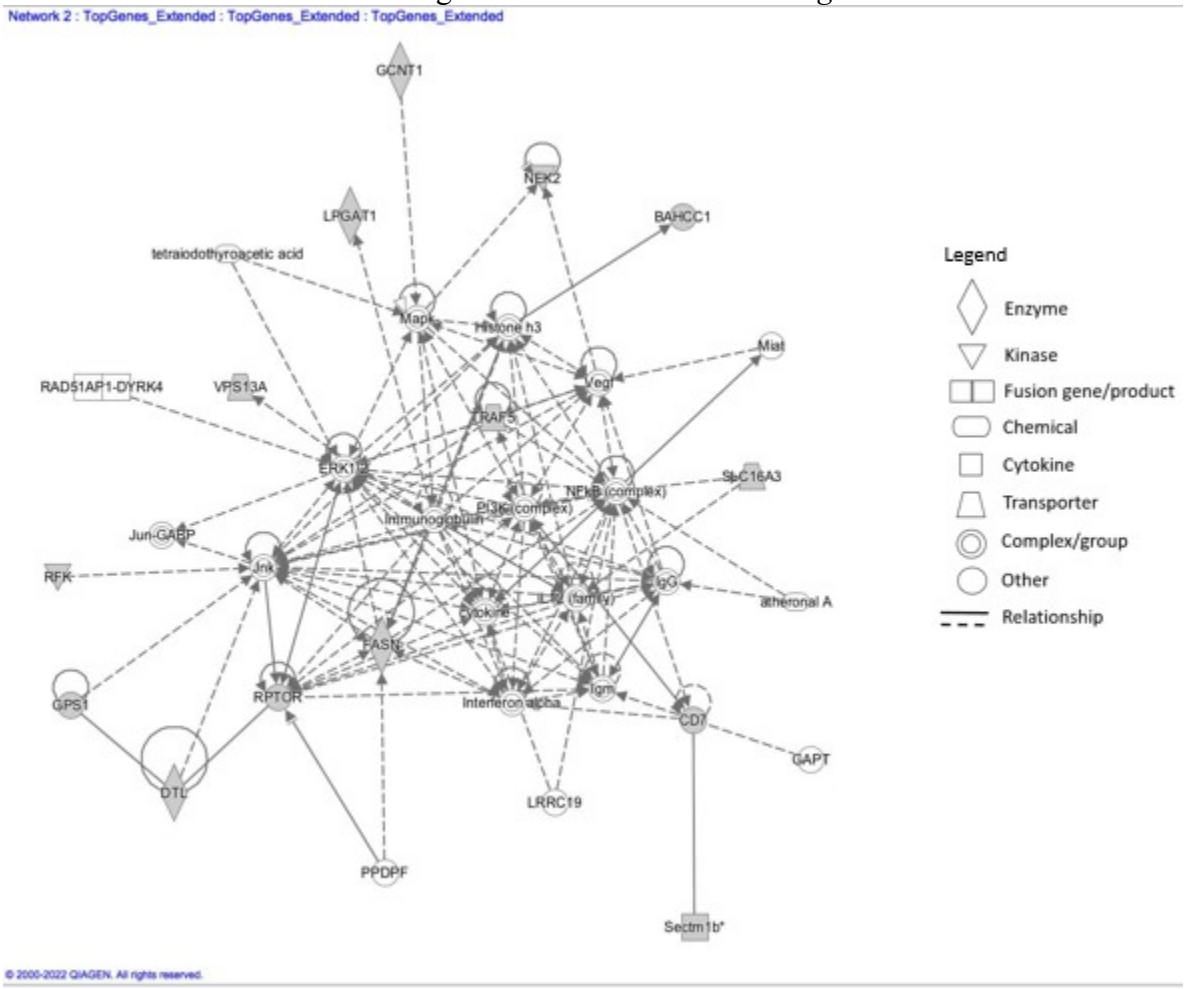

Figure 3.7: Genes identified in proximity to SNP windows that explained >1% variance for collagen characteristics in beef *longissimus* muscle, and their involvement in the cancer, cell cycle, and organismal injury and abnormalities pathway.

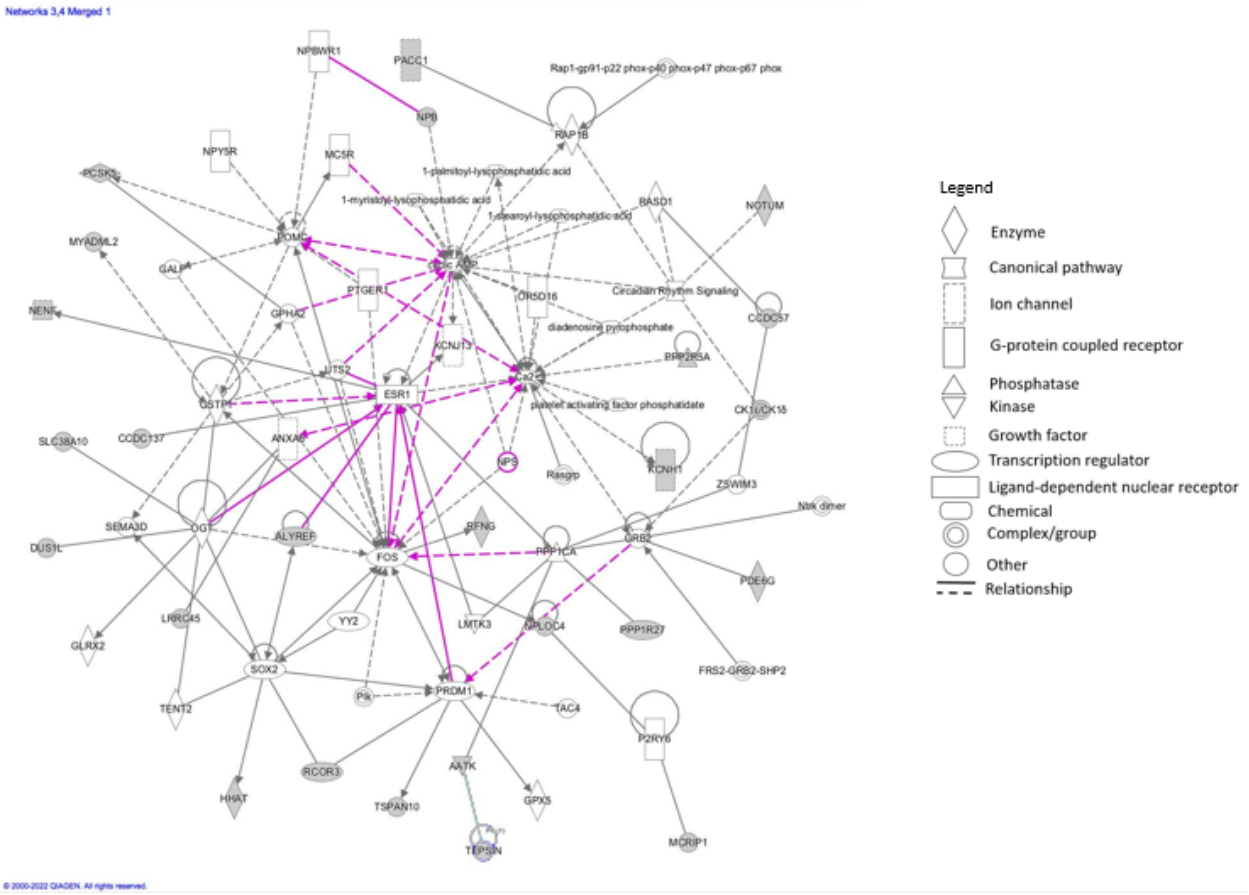

Figure 3.8: Genes identified in proximity to SNP windows that explained >1% variance for collagen characteristics in beef *longissimus* muscle, and their involvement in the cell signalling and development, molecular transport, nucleic acid metabolism, hematological system development and function, and lymphoid tissue structure and development pathways.

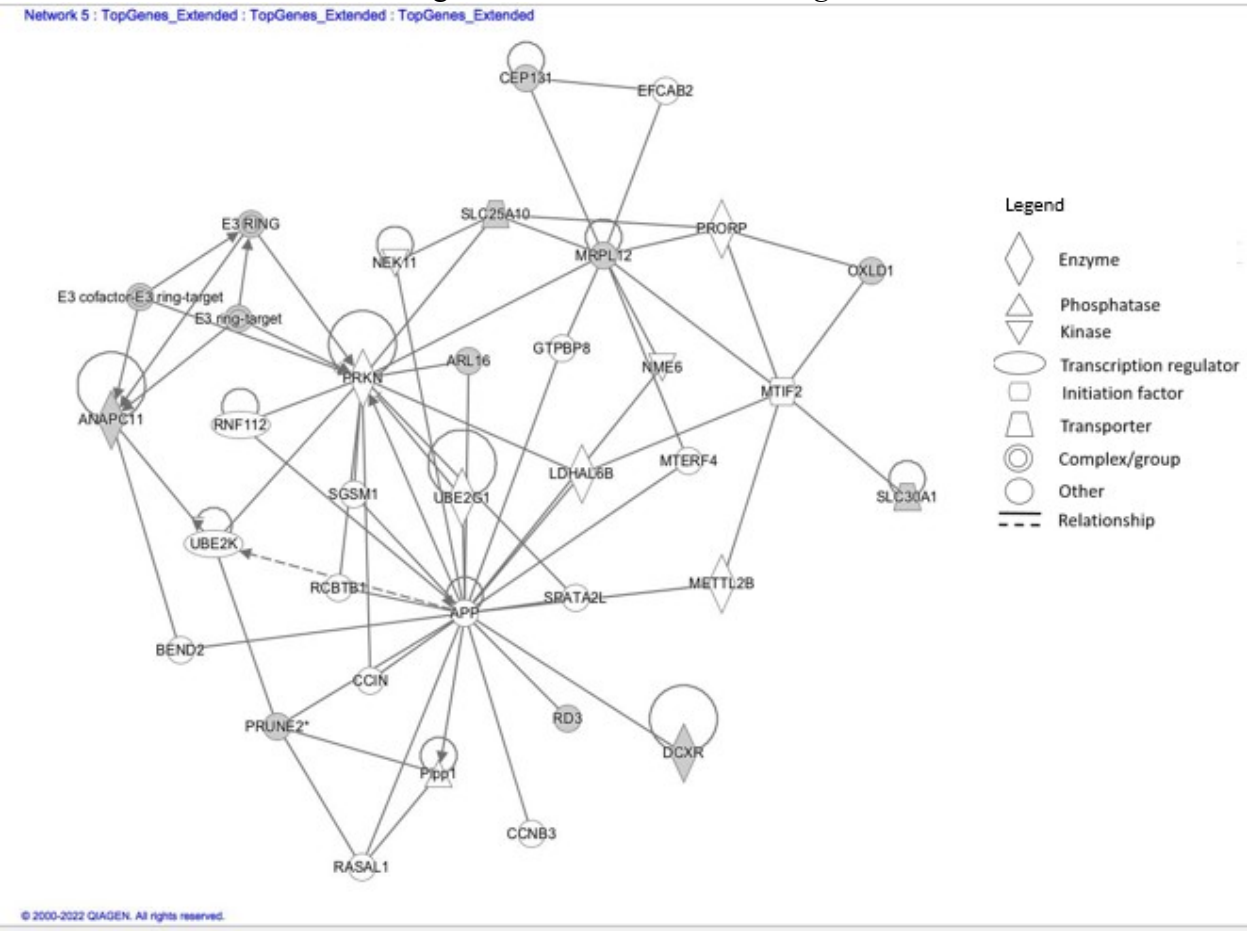

Figure 3.9: Genes identified in proximity to SNP windows that explained >1% variance for collagen characteristics in beef *longissimus* muscle, and their involvement the cell morphology, function and maintenance, death and survival pathway.

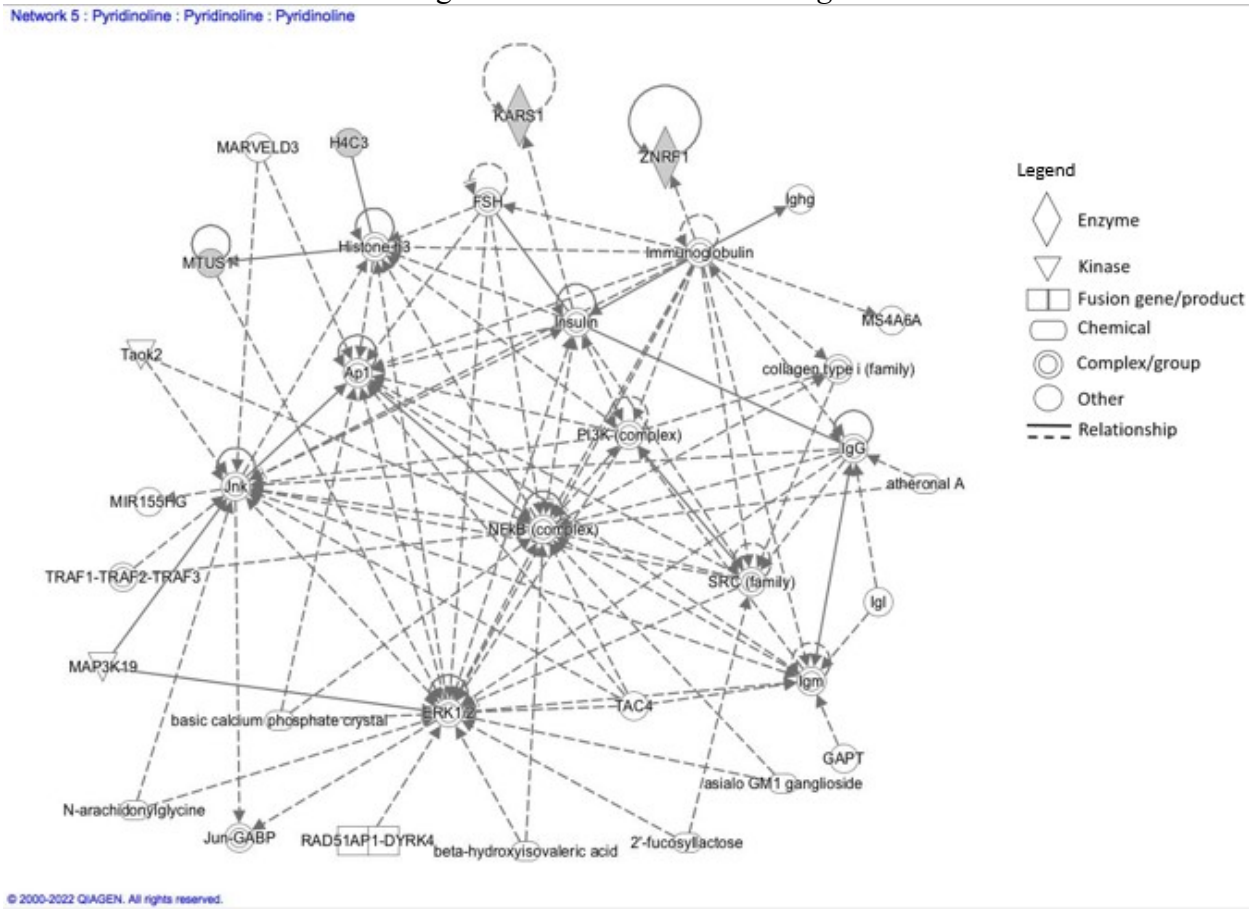

Figure 3.10: Genes identified in proximity to SNP windows that explained >1% variance for pyridinoline crosslink content in beef *longissimus* muscle, and their involvement in the organismal injuries and abnormalities pathway.

Chapter 4: Discussion

4.1 Quality and yield grade

In Canada, yield grade refers to the percentage of the animal carcass that can be sold as meat, while the quality grade is a grade given based on multiple factors that are assessed at slaughter and during carcass fabrication. These factors include animal maturity, sex, and muscle conformation, the colour, texture, and marbling of the longissimus thoracis muscle at the 12th and 13th rib interface, and the colour and amount of intramuscular fat, as well as the thickness of the fat cover of the longissimus thoracis. By their criteria, the differences between the quality and yield grades are compositional, and so it is therefore expected that relationships will exist between these two categories. Also, we expect to see certain results for quality grade and yield grade that are related to carcass and muscle composition.

In the present study, yield grade improved (approached Yield Grade 1) as quality grade decreased (approached Canada AA). This is expected as quality grade improves with increased fat, and animals that tend to produce more muscle and therefore have a better yield grade tend to be leaner, and thus have a reduced quality grade. This is consistent with the findings of Kononoff et al. (2005) where single nucleotide polymorphisms (SNPs) influencing leptin mRNA levels in adipose tissue and fat deposition in beef were alternatively associated with quality and yield grades, where one form of the SNP tended to be present in highly marbled beef, but an alternate form dominated when beef yield grade was higher.

This relationship also goes on to explain trends in the meat quality data of the present study. As quality grade approached Canada Prime, backfat and marbling score increased, while dressing percentage and LT area decreased, and those relationships were inverted for yield grade, with numerically higher yield grades showing higher backfat and lower dressing percentage. Canada Prime LT samples had less lean meat and more fat, while leaner LT was from carcasses that had yield grades of 2. Shear force decreased with quality grade while shear values increased with yield grade, with tougher LT coming from animals with better yield and lower yield grade values. This is consistent with the findings of Listrat et al. (2020) where tenderness and intramuscular fat were positively correlated. In the 2020 Listrat study, meat tenderness when evaluated by a trained panel tended to increase as intramuscular fat increased in the four muscles that were measured, and the difference in tenderness was significant for the longissimus thoracis muscle, which had the most marbling. It was noted that this tends to be the case at higher levels of marbling, and that meat tenderness does not differ due to intramuscular fat when the quantity of marbling is low, which is consistent with the lack of a significant difference in meat tenderness for muscles in the study with a low fat content. As quality grade is determined by measuring characteristics of the longissimus thoracis, however, the correlation between tenderness and marbling of that muscle in this study is more relevant to the relationship between quality grade and meat tenderness. Indeed, fattening animals for the same amount of time and to the same measured levels of fatness can reduce the difference in tenderness that arises due to breed differences (Jurie et al. 2007), indicating that muscle fatness and meat tenderness are correlated in beef.

Both yield grade and quality grade had some correlations with colour. Lightness was higher for LT from Canada Prime carcasses, which can be explained by previous findings that

associate meat colour with intramuscular fat content such as the 2006 study by Chartrin and colleagues where it was found that higher intramuscular fat in duck breast meat leads to lighter meat than is seen in leaner samples. Although LT from carcasses with numerically low yield grades were not significantly darker than LT with numerically high yield grades in the present study, lightness did tend to decrease as numerical yield decreased. A similar trend was seen for hue, with the highest average value for hue associated with Canada Prime LT and LT from carcasses with a yield grade of 5. As hue angle is a measure of red to yellow, we can expect to see an increase in hue angle measurements as intramuscular fat increases in muscle, as fat is more yellow compared to the redness of muscle tissue. This is consistent with a previous study measuring the effects of prebiotics on meat quality of finishing pigs (Grela et al. 2021) where hue angle was higher in samples that had a greater degree of marbling. This was also found to be the case in beef where after an aging period, LT from carcasses with a quality grade of Canada Prime and Canada AAA were more yellow than grades of Canada AA and Canada A (Puente et al. 2016).

Although there were few effects of quality grade on collagen characteristics, the percentage of soluble collagen at 7 days post-mortem was highest in LT from Canada AA carcasses. This may be due to the lower physiological age of the cattle that produce Canada AA carcasses, as they will still be in the active growth phase and have less time to accumulate intramuscular fat stores. We would expect to see an increase in newly synthesized collagen in animals that produce a carcass in this grade as collagen synthesis would be at its greatest, and this newly synthesized collagen would be more soluble than mature collagen (Purslow, 2018). However, this difference between the quality grades disappeared after aging, though this may be because collagen solubility at 21 days post-mortem was measured at another location in the

muscle, therefore any differences in collagen solubility due to aging should be considered with caution due to the positional effect. It is possible that there was less of an effect of fat deposition on the integrity of the collagen structure at the location from which the aged section was taken. For yield grade, LT from carcasses with a yield grade of 2, which are carcasses that have a greater amount of saleable yield, had a greater mean percentage of collagen solubility at 7 days post-mortem than LT from carcasses of other grades, and this difference persisted even after aging when collagen solubility was measured as a percentage of total collagen from the pyridinoline assay. Carcasses with lower numerical yield grades have a lower amount of fat that needs to be trimmed, which again indicates an animal of a younger physiological age that is actively synthesizing collagen.

Pearson correlation coefficients indicated that total intramuscular collagen increased as the percentage of fat in the LT decreased, and that the percentage of collagen solubility at 7 dpm increased as the percentage of lean in the LT increased. These results suggest that muscles with less intramuscular fat have a higher amount of total collagen, and that the collagen is more easily broken down by heat. This contrasts with the findings of Bruce et al. (2022) where total collagen was higher in Canada Prime carcasses, which have a higher fat content than the other quality grade carcasses of Canada AAA, AA, and A. A study in Hanwoo beef found this to be true in other muscles as well, with total collagen being lowest in quality grades with high intramuscular fat not only for the longissimus thoracis, but also for the semitendinosus, semimembranosus, psoas major, and serratus ventralis muscles (Moon, 2006). This author did not find a trend between total collagen and collagen solubility, however, and collagen solubility did not differ due to quality grades for any of the muscles studied, which is consistent with the lack of a significant relationship found in collagen solubility after post-mortem aging in this thesis.

Quality and yield grades both showed significant correlations with breed composition. The steers in this study were a mix of purebred Angus and Angus crossbreeds, with a considerable amount of Limousin as well. Angus genetics were associated with the Canada Prime and AAA quality grade carcasses, as well as numerically high yield grades. Angus is a breed that has been found to marble more readily than others and tends to yield fatter carcasses (Shahrai et al. 2021), which has led it becoming a dominant breed in the beef industry. Gelbvieh and Limousin genetics were associated with a reduction in numerical yield grade, as these breeds have a propensity for lean muscle growth (Wheeler et al. 2005). Limousin and Salers genetics were associated with both a reduction in marbling and a lower numerical yield grade, which is consistent with the inverse relationship between yield grade and quality grade that was observed in the present study. Previous work found that Salers when compared with Holstein cattle with the same fat score had significantly more muscle tissue, even though carcass weights did not differ between breeds (Jurie et al. 2007). This was also found to be the case when comparing Holstein and Charolais cattle (Bellmann et al. 2004) where Charolais cattle performed similarly to Salers and Limousin animals in the present study. Charolais, as a beef breed compared to a dairy breed like Holsteins, had better yield and more muscle per carcass, and less body fat, which would have resulted in a poorer quality grade had the researchers measured quality grade in that study. Angus genetics, on the other hand, correlated with an improved quality grade in the present study, though yield was poorer in cattle that had higher Angus content. When comparing Angus to Charolais, both of which are meat producing breeds, Jiu et al. (2019) reported that the semimembranosus, triceps brachii, longissimus lumborum, and the gluteus medius of Angus had a higher fat content and greater degree of marbling in all muscles, which is consistent with the findings of the present study.

4.2 Steroids

In the present study the use of steroidal implants affected carcass quality traits, but there was no effect on meat colour or on any of the collagen characteristics. In the 2017 group, the effect of implants on temperature was seen as soon as 45 minutes post-mortem, and there were significant differences between steroid treatment groups after 72 hours. Animals that received the backgrounding implant cooled significantly slower and had a higher pH after 72 hours than the control group and the group that received only the terminal implant, with the highest temperature and pH measured in LT from carcasses that received two backgrounding implants and no terminal implant. While the same trend was seen in 2018 animals implanted with trenbolone acetate, these findings suggest that estradiol benzoate had a more significant effect. Implanted carcasses also tended to have a lower quality grade, with the group that received two estradiol benzoate implants tending to have the lowest quality grade of all the groups, and LT from implanted carcasses were tougher, with the group that had both a trenbolone acetate implant and two estradiol benzoate implants returning the highest shear force values. Fewer effects were seen in the 2018 group, where the use of a terminal trenbolone acetate implant had no effect on pH or temperature at 45 minutes post-mortem, but the carcasses from the control group cooled more and had a lower pH than the treated carcasses. This may be due in part to the difference in muscle mass as the treated carcasses, which cooled more slowly, had a lower numerical yield grade, and the higher muscle content may have caused the internal temperature to lower more slowly. These results are consistent with the findings of Meale et al. (2021) where carcass temperature at 72 hours post-mortem tended to be lower in highly marbled carcasses that had lower yield. In these animals, the pH decline was not associated with chilling rate, but other

work has shown that pH decline is greater in carcasses that cool at a slower rate (Aalhus et al. 2001).

Previously, it has been seen that steroid use has an adverse effect on quality grade, but positively impacts yield grade. López-Campos et al. (2013) found that animals who received an estradiol benzoate implant yielded carcasses with less marbling and therefore a lower quality grade. The literature suggests that implanted animals grow faster and have a higher final slaughter weight (Bruns et al. 2005; Folmer et al. 2009), although Ohnoutka et al. (2021) found this does not necessarily translate into a higher yield grade. This is in contrast with the findings of the present study however, as the yield grade of animals that were treated with trenbolone acetate did differ significantly from the control animals in the 2018 group. Cleale et al. (2013) examined the effects of trenbolone acetate in combination with estradiol benzoate, as well as both implants on their own compared to a sham implant control. Marbling score was lowest for the combination treatment but not significantly different between the control and single implant treatments, and despite the difference in marbling score the quality grade did not differ between groups. The results of the present study were similar, with most treatments having similar quality grades and only the cattle that received two estradiol benzoate implants differing significantly from the control cattle. Yield grade also did not differ, though dressing percentage was significantly higher in the combination treatment group. This is similar to the findings of the present study where yield grade did not change in the 2017 steers where there were multiple steroid treatments but did differ when a single trenbolone acetate treatment was compared to the control cattle. Despite the difference in yield grade not being significant in the Cleale et al. (2013) study, these results still support the findings of the present study as dressing percentage was found to differ between treatments.

The effect of implantation on shear force is well studied but inconsistent. Previous evidence (Foutz et al. 1997) suggests that cattle implanted with a single dose of estradiol benzoate and trenbolone acetate will yield more tender meat than cattle that were reimplanted with trenbolone acetate 58 days later. This supports the present findings, where the animals from the PE2T group that received the greatest number of implants produced the toughest meat, were significantly tougher than the control animals and tended to be tougher than other treatment groups. The control groups did not significantly differ from the animals that received only a terminal implant, but this is also consistent with previous work (Hawkins et al. 2004) where the tenderness of implanted animals did not differ from their non-implanted counterparts. Barham et al. (2003) found that despite initial differences in shear force values of implanted and non-implanted steers, there was no detectable difference in meat toughness after an aging period of 21 days. This suggests that the negative effect of steroidal treatment on meat tenderness can be mitigated with sufficient aging.

There were differences seen in muscle temperature and pH between the steroidal treatment groups. In the 2018 batch, pH and temperature at 72 hours post-mortem were higher for implanted animals, and carcass temperature and pH at 72 hours post-mortem were highest in the animals that received two estradiol benzoate implants in the 2017 batch. Previous evidence suggests that carcass cooling is related to subcutaneous fat in beef (Seaman et al. 2002), and a study comparing bison and cattle meat found that pH was lower in samples that had lower marbling and better yield grades (Roy et al. 2020). Similar results were seen in the present study, where samples tended to have higher pH values and slower cooling, and therefore higher temperatures, when they had better quality grades, and therefore fattier carcasses, and numerically higher yield grades, which indicate a poorer yield.

4.3 Meat quality and collagen characteristics

There were various correlations between meat quality characteristics and collagen characteristics in the present study, though these correlations were not always consistent across the three groups considered. In the Guelph animals, the percentage of soluble collagen at 21 days post-mortem increased with subcutaneous and intermuscular fat, while for Lacombe animals born in 2018, collagen solubility was associated with age at slaughter, crude protein percentage, temperature, and yield and quality grades. Total collagen increased as the fat percentage of the LT decreased for the Guelph samples, while total collagen was associated with meat lightness scores in the Lacombe 2018 cohort. In summary, collagen characteristics were significantly correlated with fat characteristics, and other relationships such as correlations with meat colour and carcass temperature likely are due to the relationships those characteristics have with fat and marbling. However, collagen characteristics did not have an effect on shear force of the LT for any of the groups in this study.

Work in horse meat suggests that adipose tissue and therefore fat content of an animal may disrupt the collagen network, leading to a weaker collagen structure and increased solubility (Roy et al. 2018). In the present study however, collagen solubility was positively correlated with muscle leanness, and total collagen increased with LT leanness, and decreased with LT fatness. The findings for total collagen contrast those of Christensen et al. (2011) where breeds with medium to high levels of body fat also had a relatively high amount of total collagen. However, this may have been a breed effect and not reflect the relationship between intramuscular fat and collagen characteristics. Collagen solubility in the present study did tend to increase with muscle leanness, which is in agreement with the findings of Christensen and colleagues, as they found that insoluble collagen increased with intramuscular fat. A recent study

on the effects of different periods of grazing prior to finishing on a concentrate diet saw results that most closely resemble the findings of the present study (Mezgebo et al. 2019). The group that grazed for the shortest period of time before going onto a concentrate diet were leaner, had more highly soluble collagen, and had lower levels of collagen crosslinking, which may have had an effect on collagen solubility. This suggests that collagen characteristics may be heavily influenced by the growth rate of the animal. Total collagen, however, did not differ between the treatment groups in this experiment. The association between total collagen and lean muscle in the current study may be due to the dilution of collagen by the increase in intramuscular fat.

A 2002 review by Weston and colleagues found that collagen crosslinks accumulate with age and decrease the solubility of collagen, which may explain the association between collagen solubility and age in the present study. In this case we might expect to see an increase in collagen crosslinks with age at slaughter, but the amount of pyridinoline crosslinks present only tended to increase with slaughter age. There may have been a more significant relationship between crosslinks and slaughter age had age at slaughter been more varied between the animals of the present study. A more recent article found that collagen solubility was lower in older animals when comparing bulls at 16 and 19 months (Nian et al. 2022).

Collagen characteristics have been inconsistently associated with shear force and tenderness. Nian et al. (2017) found that meat tenderness was positively correlated with collagen solubility in the LT of male dairy cattle, however intramuscular fat content was also associated with lower WBSF values, and intramuscular fat may have increased collagen solubility which confounded collagen solubility's influence on meat tenderness. Age also was not associated with collagen characteristics, contrary to the findings of the present study. Further evidence suggests that collagen does not have an effect on meat tenderness in the *longissimus lumborum*, which is

in agreement with the present study (Holman et al. 2020). Starkey et al. (2016) suggests that this inconsistency may be due in part to the amount of collagen in a given muscle. Their work with lambs found that collagen solubility increased the tenderness of biceps femoris of lamb increased, but collagen solubility did not affect the tenderness of the semimembranosus or longissimus muscles, which tend to have a lower collagen content.

4.4 Multiple regression

It is not possible to determine a single regression equation for shear force as the models for describing shear force values differ between years of production (2017 and 2018 for Lacombe samples) and between facility. One variable that was present in all regression equations however was cooking loss. Each regression equation also had a colour measure as a variable, though what measure was most significant and therefore included in the regression equation differed between the three equations. The regression equations for the Lacombe samples in 2017 and 2018 were similar in that they both had cooking loss and age at slaughter as variables, and both also had a colour measurement, these being LT chroma and LT b* for the 2017 and 2018 samples respectively.

Age at slaughter was associated with shear force values in animals where the age at slaughter was known. Because the age at slaughter was unknown for the Guelph samples it is not clear at this time whether or not animal age would have had an effect on the shear force values. For animals where the age at slaughter was known there was a correlation both for the animals born in 2017, where there was a large discrepancy in ages (the group was mixed calves and yearlings), and for the animals born in 2018 where animal age tended to be similar. Meat toughness tends to be affected by age at slaughter due to a variety of variables, such as lower collagen solubility and a higher density of mature collagen crosslinks in older animals (Purslow,

2018), larger muscle fibers (Tornberg et al. 1994) and lower levels of myofibrillar proteolysis, though the increase in fat (Domìnguez et al. 2015) and cooking yield that comes with age also tends to improve tenderness.

While there was a significant trend between shear force and age at slaughter, the correlation was ultimately weak, indicating that age at slaughter was a poor indicator of shear force in the LT and failed to describe most of the variation in shear force values. This was also supported by the contrasting relationships between shear force and age at slaughter between the two Lacombe sample groups, where shear force tended to increase with age for the animals born in 2017 but decrease with age for the animals born in 2018. This may be due to there having been little variation in age at slaughter for the 2018 animals. As the animals in this study were all young and of a similar age, as well as crossed with Angus, the variation in shear force values due to age may not have been as significant as it would have been had there been a larger range of age. These findings are consistent with previous studies in water buffalo (Turan et al. 2021) and beef (Moloney et al. 2020), though they differ from the study conducted by Hyslop et al. (2021), which found that the difference in peak force values between young and old steers approached significance, with peak shear force being higher in older steers, and the toughness scores given by a trained sensory panel were significantly higher for older steers. Closer inspection of this study revealed, however, that there were three different finishing systems, and that differences in toughness scores were between the oldest group of animals (31-34 months) and the two younger groups (14-16 months and 20-24 months respectively). There was not a meaningful difference in meat toughness between the short and medium length feeding systems, meaning animals from 14 to 24 months had similar toughness scores when evaluated by a panel and when measured using Warner Bratzler shear force.

Colour did have a significant relationship with shear force for all groups, though the correlation was weak. Measures of meat colour are related to one another, so while the colour measurement that was most significant in the determination of shear force values between treatments may differ, the selection of a single colour measurement is due to the stepwise elimination of insignificant or confounding variables in the multiple linear regression equation, and so the colour measurement that is included depends simply on the dataset. The weak correlation between colour and shear force is consistent with the findings of Modika et al. (2015) where there was a slight correlation between meat colour and shear force values, but ultimately colour measurements failed to capture and fully describe the variation in meat tenderness. These findings are also supported by the results of the study performed by Muchenje et al. (2008) where meat colour, specifically meat lightness differed between beef breeds while shear force values were consistent between breeds. These findings contrast with those of Chulayo and Muchenje (2016) where meat lightness increased as shear force decreased, however intramuscular fat content can cause an increase in meat lightness, and there was a significant difference in muscle fatness in the study. In the 2008 study by Muchenje et al. intramuscular fat did not significantly differ, indicating that meat lightness can be associated with changes to shear force values when the differences in muscle lightness are due to intramuscular fat content, and muscle colour is not necessarily a strong indicator of meat toughness independent of other factors. Attempts have been made, however, to assess the validity of colour evaluation in the determination of meat tenderness, with some success; the support vector machine is a technique that uses a small number of training samples to build a model by which to classify samples, and has been able to correctly predict which steak has a lower WBSF value with up to 100% accuracy (Sun et al. 2012).

Cooking loss had a significant effect on the shear force of each group, though the correlation was low as cooking loss failed to explain much of the variation in shear force. Shear force values tend to increase as cooking loss increases, as cooking loss represents the loss of moisture in meat due to cooking. As moisture leaves the muscle, the space taken up by moisture decreases, and the weight of the meat decreases. It is more difficult to shear through dry cooked meat than it is to cut through meat with low cooking loss as, in terms of mass and volume of the meat, there is more tissue to get through in dryer meat, as opposed to cutting through water in samples with a lower cooking loss. There may be a relatively low correlation between cooking loss and shear force in the present study simply because there was lower variation in cooking loss between samples. The findings of this study differ from those of Kong et al. (2008) where the tenderness of chicken breast and salmon fillets were compared at various times during high heat cooking. These authors found that, as expected, muscle shrinkage increased with cooking loss, and samples with higher cooking loss were also tougher. While not directly compared in the 2017 study by Holman et al. shear force decreased as cooking loss decreased, further contradicting the results of this study. Holman et al. (2017) were interested in the effects of frozen or thawed status on shear force measurements, however, as well as initial sample weight, and found a significant relationship between pre-cook weight and shear force. When samples were cooked for the same amount of time, larger blocks of meat were cooked more slowly, myofibrillar proteins and collagen were denatured at a reduced rate, and meat toughness was reduced, indicating that cooking loss is only one factor in measuring meat tenderness. This also agrees with the findings of Zhuang and Savage (2013) where cooking loss and shear force decreased simultaneously. Both measures were higher in chicken cooked from frozen as opposed to being cooked after thawing in a refrigerator. These authors did mention, however, that it is

unclear how applicable the findings of the study are to cooking loss and meat tenderness in red meat, as chicken breast differs from beef in texture, fat, and the ratio of different muscle fiber types, as well as in how the muscle responds to temperature changes post mortem. While cooking loss can have an effect on meat tenderness, the physiological changes that occur in meat that led to differences in cooking loss may also affect meat tenderness. In the current study where there was a weak correlation between cooking loss and shear force it becomes clear that there are multiple factors involved in meat toughness. This agrees with previous research (Obuz and Dikeman, 2003) where there was a similar lack of significant correlation between cooking loss and shear force. While cooking losses were much higher in beef that was cooked from frozen than beef that was first thawed before cooking, shear force did not differ between the two treatments, showing that cooking loss is not the only determining factor of meat tenderness.

Intramuscular fat refers to fat deposits within the muscle itself and contributes to marbling in meat. The layering of intramuscular fat (IMF) between myofibrillar fibers reduces shear force values in fattier cuts in comparison to lean meat of similar weight, as there is less muscle tissue to shear. IMF has also been found to interrupt the collagen structure of horse meat (Roy et al. 2018) which may reduce the influence of intramuscular connective tissue on background toughness. However, IMF failed to capture the variation in shear force values of horse meat and the correlation between IMF and shear force only approached significance, which is consistent with the findings of the present study. In the present study IMF tended to decrease with the increase of shear force values in samples where IMF was measured. It was included in the multiple regression equation for calculating shear force in the Guelph samples, however the correlation between shear force values and IMF content was low, as it failed to explain a large amount of the variation in shear force values. This trend is consistent with the findings of

Gajaweera et al. (2018) where sensory characteristics (juiciness, tenderness, flavour-likeness) were only significantly correlated with IMF content in bulls. It had no effect on sensory characteristics in cows as the scores varied too widely within IMF groups to be significant, and in steers IMF had an effect on tenderness scores at higher levels, but there was no difference between 5-10% and 10%< IMF content groups. Shear force was lower in steers than either bulls or cows, indicating that the maturity of the animal may have more of an effect on meat tenderness than IMF content, though IMF content was highest in steers in keeping with the trends previously seen between tenderness and fat content. The weak correlation found in the present study contrasts with previous research (Therkildsen et al. 2021) where IMF content was highly correlated with shear force within the muscle of study. However, collagen content and proteolysis also played a role in shear force, and these varied in level of importance based on the feeding phase of the animal being studied. An attempt to isolate IMF and breed as factors affecting meat quality characteristics in Angus, Simmental, Limousin and Charolais (Chambaz et al. 2003) resulted in samples from Angus and Limousin having a higher degree of tenderness when evaluated by a sensory panel. Shear force as determined using machine measures did not find a significant difference between breeds. Angus reached the high level of IMF content much earlier and was slaughtered sooner, while Limousin was raised for longer than the other three breeds as it took longer to put on fat, so animal age may have played a role on the differences in meat quality characteristics in this study. Other possible contributors to the differences in meat tenderness were theorized, such as pH values, sarcomere length, and the rate of cooling of the carcasses.

Overall while the regression equations were determined by identifying trends in the data, each equation had a weak relationship with shear force, indicating that the variables that were in

the equation, while significant together, were poor indicators of shear force and failed to capture most of the variation in the data.

4.5 Effects of aging on collagen solubility

Beef tenderness is one of the most important factors consumers consider when purchasing red meat (Robbins et al. 2003), and so much attention has been paid to increasing meat tenderness and controlling the factors that may contribute to meat texture. A major factor in meat tenderness is the structure of myofibrillar proteins, and physical stress such as tumbling (Tuell et al. 2022), as well as aging the meat to allow for the activity of proteolytic enzymes (Goll et al. 2003) have been shown to improve meat tenderness. Electrical stimulation has also been employed (Mikołajczaka et al. 2019) to improve the consistency of meat tenderness by reducing the contraction of the sarcomeres of the muscle and thereby reducing the effects of cold shortening.

Collagen plays a role in meat tenderness, though its influence is inconsistent. Collagen content may be the most important factor influencing meat toughness between muscles, and it can have an effect on all muscles, even if it is not the main driver behind meat toughness (Chun et al. 2020). In contrast, Schönfeldt and Strydom (2011) found collagen content to have no effect on meat toughness when evaluating 15 different primal beef cuts, and in fact found that the M. obliques abdominus externus, despite being very high in total collagen, was rated as very tender. Where collagen does have an effect on meat tenderness, though, content alone is not the only factor. Improving collagen solubility can decrease the effect total collagen content has on meat toughness, and more tender meat tends to have more highly soluble collagen (Ebarb et al. 2017).

In the present study, collagen solubility seemed to decrease with aging in the Guelph samples after transforming the data, though this was not seen when a non-parametric test was

used on the original data. In the Lacombe samples, collagen solubility was significantly reduced after aging in the samples from animals born in 2017 and tended to decrease with aging in the 2018 samples, though the effect was not significant. A recent study saw a decrease in total collagen with aging, resulting in a higher density of mature collagen crosslinks, which stabilized the collagen structure and would decrease collagen solubility with aging (Chun et al. 2020). The authors provided no explanation for the disappearance of collagen content with aging however, and other evidence suggests that total collagen does not change with post-mortem aging, and that collagen solubility increases as collagenases deteriorate the connective tissue network (Li et al. 2008). Palka observed the same in a 2003 study, where collagen solubility saw a twofold increase between day 5 and day 12 of post-mortem aging of the semitendinosus muscle.

It is possible that animal breed played a role in why there was not an increase in collagen solubility after aging. Proteolytic activity continues post-mortem, and this is why aging is employed to improve meat tenderness, but a 2008 study comparing Angus, Nguni, and Bonsmara cattle found that aging did not improve the tenderness of the Angus cattle, while it did decrease the shear force values measured for the other breeds in the study (Muchenje et al. 2008). As the animals in this study were Angus and Angus crossbreeds, the proteolytic potential of the collagenases may have already been realized by the time of the initial measure of collagen solubility at 7 days post-mortem. The decrease in collagen solubility may also have been due to a positional effect, as the portion of each sample that was allocated to the aging treatment was kept consistent throughout the samples, and collagen content varies throughout the muscle tissue.

4.6 The importance of collagen

When looking to purchase beef, there are many characteristics that consumers consider, such as price, colour, and fat content, but when it comes to acceptability of red meat, tenderness

is one of the most important factors in determining eating satisfaction (Robbins et al. 2003). As such, much attention has been paid to improving the tenderness of beef to provide a better eating experience to consumers. Methods of improving tenderness can be applied prior to slaughter. For example, restricting feed prior to slaughter, and then offering ad libitum feed up to slaughter can cause compensatory growth that improves meat tenderness through increasing intramuscular fat, and possibly through increasing protein turnover (Therkildsen et al. 2011). Post slaughter strategies have also been employed. Physical interventions such as tumbling have been shown to significantly improve meat tenderness by mechanically disrupting the myofibrillar structures that increase toughness (Nondorf and Kim, 2022). Aging has been found to improve meat tenderness, though it is sometimes muscle dependent, with muscles that have a lower amount of intramuscular connective tissue benefitting more from post-mortem aging than more tender muscles (Tuell et al. 2022).

Intramuscular connective tissue and its main component protein collagen are not responsive to many of the treatments that are applied to meat to improve tenderness as these methods focus mainly on disrupting the structure of myofibrillar proteins, and so collagen contributes to a 'background toughness' that must be dealt with independently (Marsh and Leet, 1966). There is, however, some controversy surrounding the contribution of collagen to the toughness of meat. A recent paper found that sarcomere length decreased and particle size increased with shear force values, while total collagen and protein solubility had no significant effect (Holman et al. 2020). Still, other researchers found that total collagen content significantly influenced meat toughness (Phelps et al. 2017), and that higher collagen solubility was associated with more tender meat (Strelzleni et al. 2007) even within the same animal. The effect of collagen on meat tenderness seems to depend largely on muscle type (Anderson et al. 2012),

therefore it is worthwhile to evaluate collagen characteristics in beef, especially for muscles that have a higher amount of connective tissue.

In the present study, collagen solubility did not increase with aging. This contradicts the findings of Li et al. (2008), where it was suggested that collagenase activity should continue post-mortem and lead to higher collagen solubility. The results of the present study may be due to the breed of the animals used in the study. Muchenje et al. (2008) found that after aging, meat from Angus steers did not increase in tenderness, unlike the meat from Nguni and Bonsmara breeds. Phelps et al. (2017) also found that the amount of Brahaman genetics in an animal had an influence on meat tenderness, and that tenderness and connective tissue had an inverse relationship, where animals with a higher amount of Brahman heritage were tougher and had more connective tissue. It is possible that the use of Angus and Angus crossbreeds in the present study resulted in samples with a low amount of connective tissue that would not benefit from long term post-mortem aging, as much of the proteolysis in the samples would have occurred very quickly after slaughter. The fact that collagen solubility seemed to decrease with aging in the present study may be due to a positional effect, such as the portion of the sample that was not aged being closer to a tendon that would result in a higher amount of intramuscular connective tissue in that portion of the sample. The location effect was not evaluated in this thesis.

4.7 Heritability and correlations

Estimates for the heritability of the measured collagen traits can be used for genetic selection. Heritability of most of the measured collagen traits in this study were moderate to low (13.64-38.22%), indicating that the phenotypic variation of each trait is largely controlled by management/environmental factors rather than genotype. However, the solubility of collagen after the muscles were aged was highly heritable at 58.52%. This suggests that some traits post-

mortem such as collagen solubility are largely controlled by the environmental conditions at the time of slaughter; however, at the point where the carcasses are all undergoing similar treatment the genetic influence begins to play a more significant role. In the present study where the heritability of collagen solubility after aging was high (58.52%), the results suggest that collagen solubility is a candidate trait for genetic selection, and that solubility can be optimized through a combination of genetic selection and aging treatments post-mortem. This is further supported by the GWAS analysis, which identified a SNP window that accounted for 4.19% of the additive genetic variance of collagen solubility after post-mortem aging, which was the greatest amount of variance explained by a single SNP window that was found in this study for all five of the collagen traits measured.

While there have been studies that examine the heritability of animal traits in cattle, there has been little genetic study into collagen traits specifically, and so there is no literature to support the heritability estimates of the present study. There has been work done in meagre fish (Vallecillos et al. 2021) where the heritability of total collagen (expressed as a percentage of muscle) was found to be low with a high standard error, which is consistent with the findings of this thesis. In humans, the heritability of the pro-peptide of type IIA procollagen, which is used to assess the biosynthetic activity of collagen and has been found in lower quantities in patients with various forms of arthritis, was estimated to be 45% (Munk et al. 2014). The high heritability of this trait contrasts with the findings of this thesis; however this may be due to this being an estimation of the heritability of a specific type of collagen protein, rather than the heritability of a broader phenotype such as overall total collagen.

While there were some strong correlations, total collagen was highly correlated both genetically and phenotypically with pyridinoline content, where the content of the mature

crosslink was inversely proportional to overall collagen content in the longissimus muscle. Crosslinking is found to be more prevalent in older animals (Purslow, 2018), suggesting that an animal undergoing growth where the connective tissue networks must be remodelled to accommodate the myofibrillar expansion does not allow for the formation of mature collagen crosslinks. Gredell et al. (2018) found that total collagen of the longissimus muscle is lowest in mature animals. This suggests that the variation of collagen content and crosslinking are both largely due to growth and the ratio of myofibrillar tissue to connective tissue. However, the results of the study by Couvreur et al. (2019) found that total collagen does not decrease significantly with animal age when comparing cattle at 54 months of age to cattle at 95 months. This lack of difference may be due to the muscle of the animal being stable at 54 months, and so an increase in total collagen was not seen as it might be for an animal that is very young and still growing.

4.8 Candidate genes for collagen characteristics

While there have been studies that related GWAS to collagen characteristics (Dunner et al. 2013; Martins et al. 2022), collagen traits were rarely the focus of study. For example, a study that aimed to identify genes connected to meat quality traits in 15 European beef breeds (Dunner et al. 2013) was able to define a novel association between *Aralkylamine N-Acetyltransferase (AANAT)*, a gene which encodes an enzyme involved in melatonin production and which is linked to fatty acid content, and total collagen. The authors of that study also found that *AANAT* was associated with pH and that *MMP1*, a gene which encodes a collagenase, was associated with calpain activity. This indicates that collagen phenotypes are polygenic, where many genes have small additive effects on the trait measured, and the genes involved are pleiotropic, having a small additive effect on multiple different phenotypic traits. This is supported by the findings

of the current study, where the average additive genetic variance explained by the SNP windows that exceeded 1% and were considered significant for total collagen, for example, was 1.54%, with the highest amount of variance explained by a single SNP window being 2.52% and the lowest that was still considered significant being 1.14%.

This thesis aimed to identify novel genetic variants that may be worth further validation in association with total collagen, collagen solubility, and the mature crosslinks Ehrlich's chromogen and pyridinoline, and to validate the previous associations between GWAS and collagen characteristics that were found using the 137 steers in the work of Dr. Lei (2019). There were no overlapping SNP regions between any of the measured traits and no common genes between characteristics, despite there being a high degree of genetic correlation between some of the traits measured. There was no overlap between the SNP windows that explained more than 1% of the additive genetic variance of the collagen traits measured and those identified in previous research that involved the 137 steers from Kinsella. This may be due to the larger sample size, or because different SNP databases were used, and the overlap between the databases that was used in the current study identified novel significant genes.

When the SNP regions that were associated with the greatest amount of variation of the measured traits were extended 1 Mb further in order to identify genes that may be in linkage disequilibrium with the QTL, the RefSeq genes searched through Ensembl Biomart for those windows were used to visualize genetic networks in the IPA software. Of the five networks that were identified there was only one connection through the gene *NPS*, which connected cell signalling, molecular transport, and nucleic acid metabolism with cellular development, hematological system development and function, and lymphoid tissue structure and development, though it was not found to be significant to collagen characteristics in the GWAS

analysis. *NPS (Neuropeptide S)* is a protein coding gene, the product of which activates the corresponding receptor *NPSR* and induces mobilization of intracellular Ca+, as well as increasing levels of cAMP which leads to the phosphorylation of MAPK (Reinscheid et al. 2005). The association of collagen characteristics with these pathways and with *NPS* especially is likely due to the effect of cAMP on meat quality, where increased levels of cAMP lead to increased lipolysis, which may affect the structure of collagen networks. The MAPK pathway has also previously been associated with hyperplastic growth of muscle tissue in beef, which leads to more tender meat that has a higher degree of soluble collagen than tougher meat (Taye et al. 2017). Further study is needed to evaluate the effect of *NPS* expression on collagen.

Genes that were found to contribute to collagen solubility in unaged samples featured heavily in the networks identified by IPA software, as there were 56 protein coding genes found just within the SNP window that explained the greatest amount of variance for the trait. By comparison, there were no genes that were found to be significant to Ehrlich's chromogen even in the SNP window that explained over 4% of the genetic variance of the trait. After extending the SNP window up and downstream by 500 kb, the *CDH10* gene was found within the most significant window for Ehrlich's chromogen. From the IPA analysis, 2 genes (*ACTG1, FASN*) related to unaged collagen solubility, 1 gene (*DTL*) related to aged collagen solubility, 1 gene (*RFK*) from total collagen, *CDH10* from Ehrlich's chromogen, and *MTUS1* from pyridinoline were investigated further to understand their roles in collagen trait architecture and functional pathways.

ACTG1 or *actin gamma 1* encodes a γ-actin that is involved in the auditory disease, neurological disease, and organismal injuries and abnormalities pathway. This actin protein, unlike alpha actins that are involved in muscle, is one of two cytoplasmic actins, and as a

constituent of the stereocilia of the ear, mutations of this gene can lead to hearing loss at higher frequencies (Miyajima et al. 2020). *ACTG1* plays a role in cell proliferation, as mouse embryonic fibroblasts that had had the *ACTG1* gene experimentally knocked out showed a significant reduction in proliferation (Bunnell and Ervasti, 2010). While this gene knockout was not fatal, mice did exhibit stunted growth. Recent research into the effects of *ACTG1* on intervertebral disc degeneration in humans found that experimentally decreased expression was associated with a decrease in type II collagen (Wu et al. 2021). This suggests that *ACTG1*, while perhaps not critical to life, is implicated in cellular development and proliferation, and therefore may influence collagen characteristics in beef through conserved growth pathways. While *ACTG1* may be a suitable candidate for altering collagen characteristics to improve meat quality in beef, care should be taken to assure that selection for variation of genetic expression does not lead to loss of gene function, as loss of function mutations seem to be associated with stunted growth.

Relating to aged collagen solubility, *DTL (denticeless E3 ubiquitin)* is a protein coding gene whose product interacts with Cullin-RING ubiquitin ligases, regulating DNA replication in response to damage and affecting cell proliferation (Higa et al. 2006). As it is involved in degradation of various proteins in the body, and plays a major physiological role in cell proliferation, it has been studied as a possible component of cancer, and in an experiment evaluating the effects of *DTL* expression in gastric cancer, *DTL* knockdown led to reduced cell proliferation, as well as a decrease in tumor cell migration and invasion (Kobayashi et al. 2015). *FASN* (fatty acid synthase) was associated with unaged collagen solubility, and like *DTL* tends to be overexpressed in cancer cells (Chiang et al. 2007), and downregulation of *FASN* decreases cell proliferation. *FASN* is a protein coding gene whose product mainly regulates lipid metabolism and has already been studied as a possible candidate for improving beef quality.

Mwangi et al. (2022) found that SNPs in *FASN* were correlated with subcutaneous fat thickness and marbling score. Whether *FASN* directly affects collagen characteristics or if collagen solubility is related to *FASN* through lipogenesis is unclear in this thesis and requires further investigation, but *FASN* may be a suitable target for genetic selection in improving meat quality and increasing collagen solubility in beef.

Within the SNP windows that accounted for significant variance in total collagen content, the gene *RFK* was found within the region on chromosome 8 of the bovine genome. *RFK* or riboflavin kinase converts riboflavin, or vitamin B2, into flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which act as important cofactors in multiple physiological functions, (Shramm et al. 2013). Riboflavin was lower in patients with non-small cell lung cancer than in healthy individuals, and so the effects of increased riboflavin on lung cancer was tested by Yang and others (2013), who found that cancer growth was increased with riboflavin supplementation, but this growth was only achieved when riboflavin levels were far beyond what would naturally occur with a regular diet. High levels of riboflavin did increase expression of the matrix metalloproteinases (MMPs) 2 and 9, however, and as MMP-2 and MMP-9 are collagenases that assist in remodeling the extracellular matrix, *RFK* may indirectly affect total collagen and collagen network structures and may be suitable for selection in modifying collagen characteristics of meat.

CDH10 or *cadherin 10* is a protein coding gene associated with Ehrlich's chromogen in the present study. The protein encoded by this gene is a type II cadherin, which assists in the regulation of cell-to-cell adhesion like other cadherins (Tepass et al. 2001). The expression of a mutated *CDH10* gene has been found to be upregulated in metastatic gallbladder cancer (Li et al. 2017), indicating that a normal functioning *CDH10* can act as a tumor suppressor. When *CDH10*

is blocked through histone methylation, cell motility of breast cancer has also been found to increase (Casciello et al. 2020), leading to increased metastasis and a poorer prognosis for the affected patients. *CDH10* appears to limit cell motility through cellular adhesion and may affect tissue growth and signalling. While the effect of *CDH10* on collagen may not by direct, it is possible that collagen characteristics, especially growth-related traits like crosslink formation, are indirectly influenced by *CDH10* expression.

Of all the networks that were identified for the five collagen traits, only one involved genes that directly influence the formation of collagen in meat. *MTUS1*, a protein coding gene on chromosome 27 of the bovine genome was found to be significant for the variation of pyridinoline content in beef. *MTUS1 (Microtubule Associated Scaffold Protein 1)* has been found in humans to act as a tumor suppressor gene and is often downregulated in patients who exhibit malignant carcinoma in comparison to those who have tumorous growths but are not similarly afflicted with malignant disease (Zuern et al. 2009). This is achieved through the inactivation of *extracellular signal-regulating kinase 1/2 (ERK1/2)*, a gene which promotes cell proliferation (Nouet et al. 2004). In the present study one of the pathways through which *MTUS1* acts on the collagen type I gene is by the inhibition of ERK1/2, which in turn cannot activate *Nuclear Factor kappa B (NFκB)*, which has been found to be downstream from *ERK1/2* (Wang et al. 2012). *NFκB* is then unable to promote the formation of collagen type I, and previous work supports the role of *NFκB* in collagen formation, where activation of *NFκB* upregulated expression of *COL1/3*, leading to collagen accumulation (Lai et al. 2019). These findings that a tumor suppressor gene has a direct effect on collagen characteristics are in line with previous research on collagen's role in cancer progression. For example, the accumulation of collagen type I has been found to increase metastasis of tumors in breast cancer (Bracus et al. 2017),

suggesting that the upregulation of *MTUS1* may reduce metastasis by inhibiting collagen formation. The formation of collagen crosslinks and thickening of the extracellular matrix also leads to malignancy and is linked to poor patient prognosis (Maller et al. 2020).

The findings of the current study present novel associations between SNPs in the bovine genome and collagen characteristics, including genetic influences on collagen crosslinks, which stabilize connective tissue networks and have been associated with increased background toughness (Roy et al. 2015). These results should be considered with caution however, as the small sample size may have increased the significance of certain SNPs. These results are also limited by the heavy influence of Angus genetics on the population, and only two muscle types were examined, these being the *longissimus thoracis* and the *gluteus medius* muscles. These results should be validated in a larger population. It may also be worthwhile to validate these findings in beef with a greater variety of breeds and muscle types.

Chapter 5: Conclusion

In this thesis, the meat quality traits that had the strongest relationship with collagen characteristics were those associated with leanness. Collagen solubility increased in the LT from carcasses with a quality grade of Canada AA compared to Canada Prime, and in carcasses with a numerically high yield grade, indicating that leaner carcasses with more muscle have higher collagen solubility. This may be due to the relative physiological age of the carcasses with lower fat content, where muscle growth is at a phase where intramuscular fat has not yet been able to accumulate, and the synthesis of new collagen to accommodate the growth of muscle tissue has resulted in more soluble collagen with fewer stabilizing crosslinks. Leanness was also found to vary depending on the genetic breed makeup of the animal, with a higher proportion of Angus genetics being associated with quality grade carcasses of Canada Prime, indicating a positive association between Angus genetics and higher amounts of marbling in beef. Future research into the effect of cattle breed on collagen characteristics in association with quality and yield grade is needed to corroborate the effects suggested by the results of this study.

Muscle growth that is stimulated by growth promotants was not sufficient in this study to show a difference in collagen characteristics between steroid treatment groups. This indicates that, while the effects of implants on meat quality traits should be considered when artificially promoting growth, collagen characteristics will be unaffected in the LT of treated carcasses. The samples in this study were all the same muscle type however, and the effects of steroids on collagen synthesis and stability may differ in muscles that have higher collagen content than the LT.

Meat toughness was not found to be affected by collagen traits. Instead, even in a population with a small age difference between steers, toughness was affected by animal age at

slaughter. Shear force values were also lower in animals with a greater amount of marbling in the Guelph cohort, and marbling will increase as animals age and muscle growth decreases as fat deposition increases. The colour associations with shear force values were more likely to be the result of meat traits than to have a direct effect on meat toughness, however these results suggest that meat colour may be used to estimate meat toughness when purchasing beef. While cooking loss was significant the correlation was weak. Ultimately meat toughness in the LT muscle was found to be multifactorial, though the results of this study should be considered with caution when applying these findings to other muscles, especially those with a higher amount of intramuscular connective tissue, where collagen characteristics play a major role in meat toughness.

Collagen solubility did not increase with post-mortem aging. In fact, collagen solubility significantly decreased in the Lacombe steers born in 2017. However, while the decrease was statistically significant, in reality the difference in solubility is negligible, as the collagen content of raw muscle is low enough that a difference in solubility as seen in this study would only be detectable using the methods employed for the purposes of this research. Given that the cattle used in this study were crossbred Angus steers, it is likely that the lack of an increase in collagen solubility with post-mortem aging is due to the tendency of Angus cattle and Angus crossbreeds to age quickly post-mortem, and that most of the proteolysis that affects meat tenderness would have already occurred by 7 days post-mortem. There was likely a positional effect as the portions of each muscle used at 7 and 21 days were from different locations on the muscle. Finally, the LT muscle samples that were used for this study had a relatively low collagen content. Future research should examine the effects of post-mortem aging on collagen solubility in muscles that

have a higher amount of total collagen, and the steaks chosen for post-mortem aging should be randomized between samples to negate a possible positional effect.

The analyses performed in this study found that collagen crosslinks and total collagen are moderately heritable traits, and that while collagen solubility prior to aging shows low to moderate heritability, the heritability of collagen solubility increases with post-mortem aging. This shows that while environmental effects dominate in the early stages after slaughter, genetic selection may be used in combination with post-mortem treatments to reduce the effect of collagen on toughness in beef. Pyridinoline was also found to decrease as total collagen increased, both phenotypically and in terms of genetic correlations, which suggests that a period of growth that encourages collagen synthesis prior to slaughter may improve meat tenderness by destabilizing collagen networks which contribute to background toughness. These results confirm the associations found between yield grade, quality grade, shear force, and collagen characteristics described above.

The analyses performed in this study also identified 5 SNP windows that explained more than 1% of the genetic variance of collagen solubility of unaged samples, 8 SNP windows for aged collagen solubility, and 5 SNP windows explained more than 1% of the genetic variance of total collagen. There were 4 SNP windows that explained more than 1% of the genetic variance of Ehrlich's chromogen crosslink content in collagen, and 10 SNP windows were identified for pyridinoline. Protein coding genes were identified within these SNP windows that are possible candidates for selective breeding to improve the collagen characteristics of beef and reduce the detrimental effects of collagen on meat tenderness, however these results need to be verified in a larger group of cattle before being included in a marker-assisted selection panel. These genes should also be evaluated for their possible effects on meat quality characteristics. As collagen

solubility after aging of samples was shown to be highly heritable, the results of this study suggest that genetic selection in combination with post-mortem aging can optimize meat quality and tenderness, and that the influence of other collagen characteristics on background toughness can be well managed prior to slaughter. The results of the functional analysis also demonstrated a relationship between collagen characteristics and tissue growth as well as cellular proliferation and organismal repair, suggesting that the genes involved in collagen synthesis are pleiotropic, and that collagen phenotypes are influenced by animal growth and the formation of muscle. However, the SNP windows that were identified in this thesis did not overlap with those that were found to be significant for the 137 animals that were part of a previous study. This may be due to the larger sample size that was used in this thesis, or it may be due to the fact that different SNP databases were used and the overlap between the databases that was used to validate previous results identified novel significant genes. The findings of this thesis should be validated in a larger population.

In conclusion, meat toughness is multifactorial, and in muscles with a low amount of connective tissue, muscle toughness is largely influenced by intramuscular fat. Steroids do not have a significant effect on collagen characteristics and can safely be used to increase animal growth without increasing the background toughness that is caused by collagen. While post-mortem aging was not found in the current study to increase collagen solubility, this may be due to the low amount of collagen in the muscle studied and the rapid aging that has been found to occur in Angus and Angus crossbred beef. Post-mortem aging may still be beneficial in other muscles and in other breeds, and proper aging techniques used together with genetic selection can improve meat quality, as shown by the high heritability of collagen solubility after post-mortem aging. Finally, collagen characteristics are genetically associated with genes affecting

animal growth, and while these results require further validation in a larger group of beef cattle before being used in a custom marker panel for genomic selection, this suggests that collagen traits can be modified with farming techniques that affect animal growth prior to slaughter.

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