Effect of breed type and residual feed intake on meat and collagen quality and expression of genes involved in collagen synthesis and degradation in bovine *m. gluteus medius*

by

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Abstract

Residual feed intake (RFI) is an index of feed efficiency that has been demonstrated to be moderately heritable, with cattle having low RFI values being efficient as they consume less feed for the same weight gain. This may be of financial benefit to producers, but to be fully beneficial to producers selection for low RFI should not compromise meat quality. One of the most important characteristics of meat quality is tenderness, for which the consumer is willing to pay a premium. The m. *gluteus medius* (top sirloin) is known to be moderate tough, but with post mortem ageing this muscle may have reduced toughness and may then be marketed as a tender muscle which will add value to the beef carcass. Collagen is known to be involved in determining the ultimate or background toughness of meat and this may be affected by cattle breed, feed efficiency (selection for RFI), and post-mortem ageing. Seventy-one (71) steers from three different breeds (Angus n=23, Charolais n=24, and Kinsella Composite n=24) were used to investigate the interaction between RFI and cattle breed type on meat quality and intramuscular collagen characteristics of the m. *gluteus medius* after 3 and 13 days of post mortem ageing. The results indicated that RFI did not affect meat pH, drip loss, sarcomere length and Warner-Bratzler shear force, but intramuscular collagen heat solubility was highest in efficient steers (low RFI), indicating that background toughness was reduced in low RFI steers. Intramuscular collagen heat solubility also increased with post mortem ageing, while cooked beef toughness as measured by Warner-Bratzler shear force decreased.

From the 71 steers in this study, 12 steers from each breed type (total n=36) were selected for high (n=6) and low (n=6) collagen solubility. Intramuscular RNA was isolated from the m. gluteus medius and the relative level of expression of 38 genes reported to be involved in collagen synthesis and degradation were determined and related to meat quality and collagen characteristics. Gene expression analysis indicated that genes involved in collagen biosynthesis and degradation were unrelated to heat collagen solubility. The relative expressions of LOX, FGFR1, FGF2, and SMAD6 were lower for muscles of Angus than of Charolais or Kinsella crossbred steers, while expressions of TIMP3 and ITGA1 were highest in Angus and Kinsella crossbred steers, and AKT1 was highest in Angus steers. These results indicated that beef producers can use this feed efficiency index to select for low RFI cattle without compromising meat quality and that the toughness of the gluteus medius and the contribution of collagen to it can be decreased with

13 days post mortem ageing. Gene expression levels may reflect differences in intramuscular adipose and collagen development between the breed types but further investigation is warranted to test this hypothesis.

Preface

The research described in this thesis was designed to elucidate the effects of breed, residual feed intake and their interaction on meat quality and intramuscular collagen characteristics of the m. *gluteus medius*. The study animals were from the University of Alberta Kinsella cattle herd and the study was performed in accordance with the Kinsella animal care protocol AUP00000777. The data for the experiment described in the second chapter (meat quality and collagen characteristics) were provided by Agriculture and Agri-Food Canada's Lacombe Research and Development Centre and were analyzed by me. An ethics approval was granted from a University of Alberta Research Ethics Board (Pro00054386, Date: June 24, 2015). This chapter was written and formatted for intended publication in the *Journal of Animal Science* with Dr. Heather Bruce, Dr. Manuel Juarez, Dr. Bimol Roy, Dr. Carolyn Fitzsimmons, Dr. Changxi Li, Dr. Graham Plastow, and Dr. Jennifer Aalhus as co-authors.

For Chapter Three, a subset of animals from those described in Chapter Two were used. Sixteen primers for gene expression analysis were designed by PhD student Rabaa Hamed and the rest of the genes were selected based on a review of the literature Data generation, statistical analysis and experimental design are my original work, with the assistance of Dr. Leluo Guan, and this thesis chapter was written and formatted for publication in the *Journal of Animal Science* with Dr. Heather Bruce, Dr. Manuel Juarez, Dr. Leluo Guan Dr. Carolyn Fitzsimmons, Dr. Changxi Li, Dr. Graham Plastow, and Dr. Jennifer Aalhus as co-authors.

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List of abbreviations

ADG	Average daily gain
AFT	Adjusted fat thickness
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BF	Back fat thickness
BW	Body weight
EC	Ehrlich chromogen
ECM	Extracellular matrix
DMI	Dry matter intake
FCR	Feed conversion ratio
GM	Gluteus medius
HCW	Hot carcass weight
HIF	Heat increment of feeding
IMCT	Intramuscular connective tissue
IMF	Intramuscular fat
PYR	Pyridinoline
RFI	Residual feed intake
WBSF	Warne-Bratzler shear force
WHC	Water holding capacity
TIMP	Metallopeptidase inhibitor
MMP	Matrix metalloproteinase
ITGA	Integrin subunit alpha
ITGB	Integrin subunit beta
COL3A	Collagen type III alpha 1 chain
LOX	Lysyl oxidase
FN	Fibronectin
FGFR	Fibroblast growth factor recptor

FGF	Fibroblast growth factor
SMAD	Mother against decapentaplegic
IGF	Insulin like growth factor
PLOD	Procollagen lysine2 oxoglutarate dioxygenase
P4HA1	Prolyl 4 hydroxylase subunit alpha
PITX	Paired like homeodomain
AKT	Serine/threonine kinase
GSK3B	Glycogen synthase kinase 3 beta
FOXO	Fork head Box

Chapter 1
Literature review

1.1 Introduction

The profound nutritional value of meat and ensuring steady access to it was most likely the impetus for animal domestication and the development of animal husbandry, with advances in animal production practices eventually leading to the production of a dependable and uniform supply of meat. The species that have become dependable, domesticated sources of animal protein in North America can be divided into two categories, the red meat (cattle, pigs, goats, and sheep) and the white meat (poultry) species. Red meat and the livestock that produce it are an important market in Canada generating $21.1 billion in revenue in 2018 (Government of Canada 2019). In Alberta, 45% of beef produced is exported, and beef production accounted for $2.75 billion in revenue to Canada in 2018 (Canadian Cattlemen's Association 2013). Beef cattle and the meat they produce are among the most important agricultural assets in the province of Alberta, with about 41% of the total Canadian inventory of beef cattle and calves located in this province (Government of Canada 2019). Raising cattle is an expensive proposition, requiring the provision of feed which represents approximately 75% of the total cost of beef production (Nielsen et al. 2013). Cattle producers are facing new challenges such as environmental climate change and more restrictive environmental legislation (Kenny et al. 2018), prompting beef enterprises to use environmentally sustainable practices to reduce production costs and increase the profitability of the business without compromising meat quality. Selection of beef cattle for low residual feed intake (RFI) is one strategy that may increase production efficiency and sustainability by decreasing feed requirements and methane production of cattle (Herd et al. 2003; Basarab et al. 2013). The term residual feed intake (RFI) was defined by Koch et al. (1963) as the actual minus expected feed intake, with feed intake meeting the animal's nutritional requirements for maintenance and growth (Savietto et al. 2014). Selecting cattle for RFI status has been shown to decrease total feed intake over the production life of slaughter cattle (Richardson et al. 1999), and has become an important selection criterion in Australia and Canada. The impact of selection for RFI on beef quality is equivocal as some researchers have found no effect of RFI on beef quality (Hunt

et al. 2006; Fidelis et al. 2017), while others have found selection for low RFI increases beef toughness (Herd et al. 2018). For this reason, additional research investigating the effects of RFI status on beef quality is warranted. The possibility exists that breed may interact with RFI, although Flay et al. (2019) found no interaction between breed and RFI for methane production by dairy heifers, and Elzo et al. (2009) found no influence of breed on growth and residual feed intake for Angus, Brahman and Angus x Brahman cross slaughter cattle. The effect of breed and its interaction with RFI status on beef quality, however, does not appear to have been investigated. To understand the research performed to date, this literature review was conducted to identify mechanisms contributing to the development of beef quality and how beef quality may be affected by breed and selection for low RFI.

1.2 Feed efficiency indexes

Measuring feed efficiency requires combined consideration of feed intake (the input) and production (output). Multiple indexes are used to quantify feed efficiency but it is important to use the appropriate index (Gibson 1986). The feed efficiency index (gain to feed) has been utilized historically, but there are other indexes that can be used, with every index having a different application (Berry and Crowley 2013). Some common feed efficiency indexes are gross efficiency, maintenance efficiency, and partial efficiency.

One of the first indexes used as a measure of feed efficiency was the feed conversion ratio and this index standardizes animal feed intake against animal weight gain during regular production practices (Nkrumah et al. 2007). This measurement provides a quick estimation of efficiency that allows comparison across animals, and is used routinely in feedlots to estimate animal efficiency (Beef cattle research council 2019). Selection for feed conversion ratio (feed to gain), although successful, can also indirectly select for an increase in mature animal size (Varkoohi et al. 2010). Increasing mature animal size will ultimately increase overall breeding herd feed consumption, as big animals will consume more feed than small animals.

Other methods to measure feed efficiency examine feed inputs needed to support maintenance and growth requirements separately. The first method is quantification of maintenance efficiency, which is defined as the ratio of body weight to dry matter intake (DMI) when body weight remains unchanged (Ferrell and Jenkins 1985). For this measure to be useful, cattle must maintain a constant weight for up to two years to get a real measure of their individual maintenance requirement (Archer et al. 1999). The second method is calculation of partial efficiency, which is average daily gain (ADG) divided by dry matter intake minus DMI

needed for maintenance (Li et al. 2016), and this index is used to estimate the efficiency of energy gain and energy loss (Veerkamp and Emmans 1995). For both methods the prediction of DMI for maintenance are derived from National Research Council (NRC, 1996) standards. A third approach uses statistical regression to predict an animal's expected feed intake, with a model based on the performance of a set of animals similar in weight and growth rate over a test period (Carstens and Tedeschi 2006).

In beef cattle, RFI has been demonstrated to be a useful index for feed efficiency (Archer et al. 1999). This index is preferred for the selection of cattle and DMI is used as the measure of feed intake (Savietto et al. 2014). Many studies suggest that this characteristic can be inherited by beef cattle (Mrode and Kennedy 1993), with the expectation that animals with low RFI values (efficient) will achieve the expected growth with a lower DMI (Moore et al. 2013).

Variation in efficiency also is due to differences in the intake of feed, in the completeness of digestion of the feed, in the metabolism associated with digestion of the feed, animal activity level, and thermoregulation (Herd et al. 2004). The variation in efficiency as measured using RFI is due to different factors, and an estimated 9% of this variation can be attributed to the heat increment of feeding (HIF), with differences in digestion usually accounting for 14% of the variation (Richardson et al. 1996). As a result, selection of the best feed efficiency index is a priority for beef producers, and use of RFI offers the advantage of increasing feed efficiency without indirectly reducing animal physiological age and increasing mature size (Varkoohi et al. 2010).

1.3 Meat quality measures of beef products

Traditionally, meat quality is associated with our sensory perception: appearance, color, texture (tenderness, juiciness), flavor and odor (Purslow 2017). Other external factors can also affect meat quality in beef products and include price, animal welfare, environmental sustainability and packaging, which can alter the consumer perception of meat quality (Hocquette et al. 2012). Laboratory-based methods for assessing meat quality have been developed to characterize meat attributes such as intramuscular pH, proximate composition, water holding capacity (WCH) and Warner-Bratzler shear force and these measurements have become indispensable for describing meat characteristics (Allen et al. 1998).

1.3.1 Warner-Bratzler Shear Force

Shear force methods in general measure the force required to shear, penetrate, compress or stretch meat (Szczesniak 1963). The Warner-Bratzler shear force method was proposed in the 1920's by K.F. Warner and colleagues (1952) and improved and modified by L.J. Bratzler, who changed the speed, the shape of the blade, and the thickness of the sample (Bratzler 1932). To get reproducible results and to reduce variability in toughness measurements samples without excessive fat and connective tissue that are representative of the whole steak are used (Boleman et al. 1997). The American Meat Science Association (AMSA) research guidelines (AMSA, 2015) contain the most widely used protocols and indicate that the blade should be 1.1684 mm thick and have an angle position of 60˚ with a half-round cutting edge. Also, a 1.27 cm diameter core is advised and that the steaks be chilled overnight prior to the shear force analysis.

1.3.2 Meat pH

After exsanguination, oxygen supply to the muscle is lost and so adenosine triphosphate (ATP) is no longer generated through aerobic metabolism. ATP is necessary to maintain muscle homeostasis, and so the muscle shifts to anaerobic glycolysis to generate ATP. With this, hydrogen ions accumulate and this leads to a decrease in muscle pH (Przybylski and Hopkins 2015). The effects of lowered pH occur early postmortem, causing a decrease in water-holding capacity (WHC), increasing muscle redness with exposure to oxygen, increasing cooking loss and reducing protein functionality (Kim et al. 2014). During the rigor process, muscle pH is between 7.0 and 7.2 (Honikel 1986) and declines to 5.3 to 5.8 with anaerobic glycolysis, with the final pH known as the ultimate pH. The ultimate pH value is reached within 18 to 40 h post mortem in beef carcasses (Smulders et al. 1992). Abnormal pH decline in postmortem muscle can lead to a decrease in meat quality and is often associated with the development of dark, firm and dry (DFD) meat (Przybylski and Hopkins 2015). Therefore, measuring meat pH during the conversion of muscle to meat during the early postmortem period and the ultimate pH of meat is important to understanding the potential color of the beef and subsequent carcass shrinkage, and meat drip loss and texture.

1.3.3 Water holding capacity

Water holding capacity (WHC) is usually indicated by the amount of water lost from meat before or after it is cut. The WHC of meat is a concern as drip loss reduces the weight of meat during processing, transportation and storage, and often occurs after cutting because muscle cells are damaged and the

cytoplasm is then free to escape. It also occurs during thawing and cooking, as water escapes when muscle cells are damaged by ice crystals or heating, respectively. WHC can affect the tenderness, juiciness and color of meat (Gault 1985;Hughes et al. 2014), and is associated with nutrient loss and reduced carcass yield (Huff-Lonergan and Lonergan 2005). Drip loss occurs when the muscle cell loses the ability to hold free water, which is the water that is held by weak electrostatic interactions, and is termed purge loss if it happens while packaged (Huff-Lonergan and Lonergan 2005). After development of rigor, when muscles convert to meat and sarcomeres shorten, the water inside the muscle cell can be expelled by the shortening of the sarcomeres as free water is displaced by the contraction of the myofibrillar proteins (Bendall 1973). Also, reduction in sarcoplasm pH approaches the isoelectric point of myofibrillar proteins (pH 5.0), which reduces the ability of myofibrillar proteins to bind water molecules (Barbera 2019). WHC also can predict cooking losses, as it can indicate how much water is covalently bound to muscle proteins (Heymann et al. 1990) and not evaporated or lost during denaturation of the proteins (Cheng and Sun 2008), shrinkage of the collagen fibers and contraction of the sarcomeres (Rao et al. 1989) during cooking. Inability to control WHC by the meat industry is associated with loss of profitability every year (Huff-Lonergan and Lonergan 2005). Hence, it is imperative to reduce the water loss postmortem and during further processing by monitoring it to ensure an optimal product quality. Drip loss or purge loss are the objective measurements needed to assess the amount of moisture loss from raw meat (Huff-Lonergan and Lonergan 2005).

1.3.4 Sarcomere length

The basic contractile unit of muscles is the sarcomere, and the sarcomeres will contract as the muscle enters into rigor post-mortem. The extent of sarcomere shortening depends upon the rate of pH decline, the temperature at rigor and the presence of mechanical restraints on the muscle, with shortening minimized by rigor occurring at 15 °C at a pH of approximately 6.0 in muscle attached to bone. Long sarcomeres are present in muscles that relaxed or stretched when they enter rigor hence, are more tender after cooking (Locker 1960). Sarcomere length is permanently set at rigor, as the interaction between myosin and actin requires ATP to be disrupted (Ertbjerg and Puolanne 2017). Tough meat is produced when the sarcomere length is short and the associations between the thick and thin filaments extensive (Warris 2000). Sarcomere length is usually measured directly by examining prepared myofibers under the microscope or by optical diffraction (Voyle 1971). Sarcomere length can be a tool to determine if cold

shortening is contributing to meat toughness, but it is most valuable when considered in combination with shear force or sensory analysis to explain the cause of meat tenderness or toughness.

1.3.5 Proximate analysis

Proximate analysis is a series of analyses that collectively describe the composition of meat in terms of its percentage of water, protein, fat and ash. These values are important because researchers can evaluate the role of beef in the diet by knowing its composition and inform consumers about making healthy selections (McNeill et al. 2012). With all the data from the proximate analysis, researchers can estimate cooked nutrient values. The analyses are standardized by the Association of Official Analytical Chemists (AOAC 1997). The sample preparation is essential for the analysis so that the result will be accurate and comparable across laboratories and between studies.

1.4 Effect of RFI on carcass and meat quality

Residual feed intake is a moderately heritable trait in beef cattle, and selection of cattle based upon RFI may affect carcass and meat quality because low RFI animals consume less feed than inefficient animals and this may change whole body energy partitioning and muscle metabolism (Fidelis et al. 2017). The selection for low RFI has also been associated with reduction in subcutaneous fat (Herd et al. 2018) and increased carcass leanness (Basarab et al. 2003). Increases in hot carcass weight (HCW) were found to be associated with three different genetic markers, specifically BTB-0055733, BTB-005557353 and BTB-00557235, all of which are associated with fat thickness (Lindholm-Perry et al. 2012). These results indicated that these markers were associated with animals that had low RFI, reduced adjusted fat thickness (AFT), higher finishing weights and increased feed efficiency, and that selection for these traits simultaneously may be possible.

Other meat quality characteristics like percentage (%) heat soluble collagen have also been observed to be affected by RFI where animals with high RFI (HRFI) had lower percentage heat soluble collagen and shear force than those with low RFI (LRFI), which had comparatively tougher meat (Zorzi et al. 2013). Zorzi et al. (2013) however, did not find any significant difference among other meat quality characteristics including intramuscular pH, sarcomere length and color. No significant differences in hot carcass weight

(HCW), DMI, back fat and Warner-Bratzler shear force (WBSF) in muscles from HRFI and LRFI Angus steers (Hunt et al. 2006). Gomes et al. (2012) found no difference in initial and final body weight, average daily gain (ADG), HCW, back fat thickness or intramuscular fat, which contradicted findings by Frotas et al. (2015) and Ribeiro et al. (2012) indicating that DMI and HCW were different between low and high RFI animals.

The effects of selection for RFI on beef toughness are also equivocal. No changes in shear force were observed by Baker et al. (2006) in Angus steers between RFI groups and McDonagh et al. (2001) showed no difference in shear force of muscle from low and high RFI Angus purebred and crossbred steers. A similar study evaluating carcass traits and meat quality of Nellore bulls with differing RFI found no significant difference in shear force values also. In contrast Zorzi et al. (2013) in their study used 59 young Nellore bulls and found that low RFI animals tended to have higher mean WBSF values. Similar results were found by Smith et al. (2011) although in pigs, who found that meat tenderness may be decreased by selecting pigs with low RFI as they found a negative correlation between RFI and the amount of desmin at 7 days post-mortem in boneless center loins, which demonstrated a decrease in protein degradation in low RFI barrows during ageing. Although the preponderance of studies indicate no effect of RFI on WBSF in cattle, further examination of this effect is warranted given that beef tenderness is a critical factor affecting consumer acceptance (Miller et al. 1996).

1.5 Effect of breed on carcass and meat quality

In the last two centuries, the trend has been to raise lean, small, young cattle for slaughter (Lawrie et al. 2006). Beef cattle have more muscle and an increased muscle to bone ratio, and so beef cattle breeds are selected based on their adaptation to the environment, ability to fulfil market demand, and the ability to grow and produce meat in a particular production system (Greiner 2005), The sub-species *Bos taurus* is capable of withstanding low ambient temperatures with its long hair coat as protection but has reduced tolerance to heat (Buchanan and Dolezal 1999). The sub-species *Bos indicus* is widely used in warm areas as this sub-species as its lack of hair confers significant heat tolerance, and cattle from this sub-species usually grows slowly and reach puberty relatively late (Lawrie et al. 2006; Hammack 2008). Selection of a sub-species and a breed within a sub-species is therefore important as it may affect meat quality adversely through

reduced feed intake during hot weather leading to dark cutting beef (Ponnampalam et al. 2017). For this reason, crossing *B taurus* and *B. indicus* results in crossbred offspring that can thrive in hot weather while still maintaining increased muscularity and carcass yield.

Since the domestication of cattle, cattle producers have used different breeds to confer specific characteristics to the carcass like time to maturity, proportion of fat, and the size of the muscles, capitalizing on the genetic variation of every breed to change meat quality attributes (Kerry et al. 2002). In the Canadian meat industry, Angus and Charolais breeds are used extensively, and both are from the *Bos taurus* species (Canadian Beef Breeds Council 2019). Angus is a particularly popular breed in Canada mainly for producing a carcass with a small size but substantial intramuscular fat at an early age, while Charolais are a popular terminal cross with Angus because they produce a large, lean carcass at a later age (Canadian Beef Breeds Council 2019). Tenderness may be affected by crossing breeds from the two type of species, as *Bos indicus* is often less tender than *Bos taurus* (Koch et al. 1982). Other authors (Cuvelier et al. 2006) studying breeds like Belgian Blue, Limousin and Angus assessed meat quality and fatty acid composition with finishing diets consisting of either sugar-beet pulp or cereal. Fat differences were found between breeds with the most stable color observed in Blue Belgian cattle and this was accompanied by a reduction in cooking loss. Differences in fat were found by Pesonen et al. (2012) who found the fat content of Aberdeen Angus bulls was greater than that of the Limousin when the cattle were fed with a grass silage-grain-based rations and raised to heavy carcass weights. Moreover, Ba et al. (2013) found that not only was intramuscular fat (IMF) greater in Hanwoo than in Angus beef but so too was cooking loss. Other traits like live weight are influenced by breed depending on the degree of maturation of the animal (Barton et al. 2006). As a result, breed is often used to control and enhance carcass composition and therefore influence carcass and meat quality.

When sensory quality characteristics were assessed, Chambaz et al. (2003) and Revilla and Vivar-Quintana (2006) found differences in odor intensity, flavour and tenderness between breeds. Other traits that change with breed are collagen and collagen heat solubility, and this was observed in the Charolais, Simmental, Angus and Hereford breeds, with the heat-soluble collagen differing by breed (Cross et al. 1984). Martínez-Cerezo et al. (2005) found with lambs using the Rasa Arogonesa, Churra-local and Spanish Merino breeds, that total and insoluble collagen were higher in Churra than in the other breeds.

Similarly, Sañudo et al. (2004) found differences in total collagen and collagen solubility among four cattle breeds but only in the *Longissimus thoracis and lumborum.* In another study comparing the *Longissimus thoracis, Semitendinosus* and *Triceps brachii* from Aubrac, Charolais, Limousin and Salers cattle carcasses, there were significant differences due to breed in insoluble collagen (Jurie et al. 2005). The influence of breed on the amount of intramuscular collagen is most likely due to the difference in the accumulation of the connective tissue and myofibrillar proteins in muscle with age by breed (Boccard 1978). Differences in total collagen content of the m. *semitendinosus* have been shown to occur with breed and were attributed by the authors to the physiological differences in the two crossbred cattle types (Roy et al. 2015). Crossbreeding programs are important for enhancing palatability traits such as tenderness through modification of intramuscular fat, but that collagen characteristics can be influenced by breed indicates that the background toughness of beef may be reduced through breed selection. These results indicate that collagen heat solubility may be heritable and therefore potentially manipulated not only by breed selection but possibly through selection of individual animals with offspring showing increased heat soluble collagen or reduced heat insoluble collagen.

1.6 The function and location of intramuscular collagen

The extracellular matrix (ECM) consists mainly of the protein collagen, augmented by other components integrated into the ECM such as proteoglycans, glycoproteins and enzymes (McCormick 1994). Connective tissue holds the skeletal muscle together and to bone (Nishimura et al. 1996), and so it is one of the most abundant tissues in skeletal muscle (Purslow and Duance 1990). The skeletal connective tissues are known as the epimysium, perimysium and endomysium (Ham 1969) and they surround the whole muscle, bundles of muscle fibers and single fibers, respectively. The endomysium is the internal network of connective tissue that surrounds each muscle fiber and is connected to a middle layer of connective tissue called perimysium, which surrounds bundles of muscle fibers and represents about 90% of the connective tissue in skeletal muscle (McCormick 1999). The perimysium is connected to the epimysium, which is the layer of connective tissue that surrounds each intact muscle and connects to bone or other muscles through tendons (Mayne and Sanderson 1985). Connective tissue content varies between muscles, species, breeds and chronological ages (Purslow 2005). Elastin is also present in the connective tissue of some muscles at approximately 0.4% of muscle dry weight (Bendall 1967). Proteoglycans, which consist of

glycosaminoglycans covalently bonded to core proteins, are usually associated with skeletal muscle connective tissue and their concentration in connective tissue will vary depending on the muscle location and function (Pedersen 2011). In a review, Purslow (2002) indicated that the concentrations of different collagen types vary during embryonic development in bovine muscles suggesting that differences in collagen content are established in early development and that there is a progression of replacement of some types of collagen with growth. Muscles involved in movement usually have an increased content of connective tissue (Zimmerman et al. 1993) and as a result these muscles may be tough.

The synthesis of collagen takes place initially intracellularly in the endoplasmic reticulum like any other protein, then it is modified and assembled outside of the cell (Myllyharju and Kivirikko 2004). As with any other protein, the mechanisms of stimulation of genes and mRNA synthesis, translation and enzymatic hydroxylation, folding and formation of the triple helix, and excision occur intracellularly up to the formation of the collagen molecule, which is transported to the extracellular space after its formation (McCormick 1994). Other processes that occur during synthesis of collagen after secretion into the extracellular space are the removal of the pro-peptide extensions on the telopeptides, incorporation of the molecule into existing collagen fibrils, and then cross-linking of the molecule by lysyl oxidase to other collagen molecules to stabilize the molecule in the fibrils (Pinnell 1982). Although the process of collagen synthesis is well known, the processes associated with the establishment of collagen types and the regulation of collagen cross-link formation is not (Bruce and Roy 2019). Further studies are necessary to elucidate the process of collagen synthesis and its mechanism in order to relate those processes to effect of collagen in meat quality.

1.6.1 Collagen type classification

Collagen in beef can vary from 1 to 15% of tissue dry weight (Bendall 1967). Fibrillar, network, filamentous and fibril-associated collagens are the main families of collagen (Boot-Handford and Tuckwell 2003) found in muscle. Many types of collagen have been discovered, now totaling more than 27 different types of collagen, each arising from its own gene (Table 1-1). Collagen peptides are unique in that they exhibit a Gly-Pro-Hyp structure that repeats over the entire chain. They also are unique, along with elastin, in that they contain both hydroxyproline (HYP) and hydroxylysine (HYL). Table 1-2 shows the various collagen types and their molar ratios of hydroxyproline to hydroxylysine, which can be used to identify the various

types of collagen. From this list, type V is most common in fish muscle (Sato et al. 1998), but the largest and the most abundant types in bovine muscle are Types I and III (Purslow 2002). Other types of collagen have different amino acids and function, such as Type IV collagen, which forms a two-dimensional reticular matrix that serves to stretch and maintain muscle tissue at the level of the muscle fibre (Kavitha and Thampan 2008). Despite knowledge that many different types of collagen exist in muscle, few researchers examine the various types given the difficulty in separating and accurately quantifying the types, as pepsin digestion is necessary and it preferentially digests collagens like Type V faster than Type I and III, prohibiting conclusive results (Cheung 1987).

1.6.2 Collagen composition

Glycine predominates the amino acid residues of mammalian collagen, representing one-third of its residues (Eastoe 1967). Proline and hydroxyproline represent about 30% of the collagen amino acid residues and impart rigidity and stability to the collagen triple helix (Wu et al. 2011). Collagen content in tissues is estimated by determination of hydroxyproline because it is an amino acid that is unique to collagen and elastin (Freiberger et al. 1980). Alanine and the polar amino acids arginine, lysine, asparagine, and glutamic acid constituting 10 and 20% of the total amino acid residues, respectively. These amino acids are important to the inter-triple-chain linkages that drive collagen fibril formation (Ramachandran and Ramakrishnan 1976). Collagen is characterized by Gly-X-Y triplets in series, where X and Y can be any amino acid but are generally proline and hydroxyproline (van der Rest and Garrone 1991). Polar sequences tend to happen after the sequence Gly-X-Y is repeated five or six times along the polypeptide chain (Pearson and Young 2012).

Table 1-1. Collagen types and their genes [Adapted from Canty and Kadler (2005)]

Collagen type	Genes	Supramolecular organization in tissue	References
I	*COLIAI* *COL1A2*	Fibrils in endomysium, perimysium and epimysium of muscle, tendons, bone, skin, cornea and blood vessel walls	Chu et al. 1982 Myers et al. 1981

II	*COL2A1*	Fibrils in cartilage	Miller and Matukas 1969
III	*COL3A1*	Forms heterotypic fibrils with type 1 collagen in muscle, skin	Cameron et al. 2002
IV	*COL4AI*	Network in basement membranes	Timpl and Brown 1996
	COL4A2		
	COL4A3		
	COL4A4		
	COL4A5		
	COL4A6		
V	*COL5AI*	Forms heterotypic fibrils with types 1	Birk 2001
	COL5A2		
	COL5A3		
VI	*COL6AI*	Fine microfibrils with ubiquitous distribution	Kielty et al. 1992
	COL6A2		
	COL6A3		
VII	*COL7AI*	Forms anchoring fibrils in skin	Keene et al. 1987
VIII	*COL8AI*	3D hexagonal lattice in Descemets membrane in the eye	Kapoor et al. 1986 and Stephan et al. 2004
	COL8A2		
IX	*COL9AI*	Associated with type 2 collagen fibrils	Olsen 1997 and Shimokomaki et al. 1990
	COL9A2		
X	*COL10AI*	Mat-like structure	Kwan et al. 1991
XI	*COL11AI*	Forms heterotypic fibrils with type II	Mendler et al. 1989

	COL11A2		
XII	*COL12AI*	Associated with type I fibrils	Keene et al. 1991, Nishiyama et al. 1994, and Zhang et al. 2003
XIII	*COL13AI*	Transmembrane and possibly involved in cell adhesion	Latvanlehto et al. 2003
XIV	*COL14AI*	Associated with type I fibrils	Young et al. ,2000, 2002
XV	*COL15AI*	Specialized basement membrane	Myers et al. 1996 and Ramchandran et al. 1999
XVI	*COL16AI*	Component of specialized fibrils in skin	Kassner et al. 2003
XVII	*COL17AI*	Transmembrane component of hemidesmosome,	Hopkinson et al. 1998
XVIII	*COL18AI*	Cleaved to produce antiangiogenic fragment	Sasaki et al. 1998
XIX	*COL19AI*	Radially distributed aggregates	Myers et al. 2003
XX	*COL20AI*	May be associated with type I collagen	Koch et al. 2001
XXI	*COL21AI*	May be fibril associated	Fitzgerald and Bateman 2001
XXII	*COL22AI*	May be associated with microfibrils	Koch et al. 2004
XXIII	*COL23AI*	Transmembrane collagen in cell cultures	Banyard et al. 2003
XXIV	*COL24AI*	Expressed in tissue type I collagen	Koch et al. 2003
XXV	*COL25AI*	Transmembrane collagen pace in neurons	Hashimoto et al. 2002
XXVI	*COL26AI*	Expressed in testes and ovary of adult tissue	Sato et al. 2002
XXVII	*COL27AI*	Expressed in cartilage	Boot-Handford et al. 2003

Table 1-2. Molar ratios of hydroxyproline in collagen types from various tissues [from Ignat'eva et al. (2007)]

Collagen type/source	Hydroxyproline/hydroxylysine molar ratio
Collagen I (warm-blooded skin fibril)	11 to 18
Collagen II (porcine larynx cartilage)	4.30
Collagen II (chicken cartilage)	4.50
Collagen III (calf skin fibril collagen I)	17.0
Collagen III (human liver)	22.6
Collagen IV (human crystalline lens)	3.00
Collagen IV (ovine crystalline lens)	2.60

1.6.3 Collagen content

Prolyl hydroxylase is responsible for the translational hydroxylation of proline in collagen and elastin (Ignat'eva et al. 2007). Because of this specificity and the low levels of elastin in beef skeletal muscle, with the exception of the semitendinosus (Bendall 1967), the determination of hydroxyproline in muscle is considered to be indicative of the collagen content of muscle (Freiberger et al. 1980). A spectrophotometric method is used to measure the content of hydroxyproline and is based on the reaction of Ehrlich's reagent (p-dimethylaminobenzaldehyde) with hydroxyproline (Schwartz et al. 1985). Collagen content varies between and within beef muscles but in some cases the distribution of collagen is more homogenous, and the *sternomandibularis,* the *longissimus dorsi, the latissimus dorsi, semitendinosus,* and *gluteus medius* are examples of such muscles (Bendall 1967). Bendall (1967) found differences in the percentage of collagen content represented as dry weight between muscles depending upon whether they were involved in motion or not. Hill (1966) showed that the amount of intramuscular collagen varied with animal age and was particularly high in very young animals when muscle was still developing.

The *gluteus medius* is a muscle that has comparable tenderness to the *longissmus thoracis et lumborum* muscle (Keith et al. 1985), but only in young cattle as this collagen in this muscle becomes substantially less soluble as cattle age (Girard et al. 2012). As it is already a valuable beef muscle at retail (Bruce and

Roy 2019), maintaining a predictable level of tenderness would allow it to continue to add value to the beef carcass.

1.6.4 Collagen crosslinking

The presence of collagen types I and III (both fibrillar collagens) and others such as types V and XI contributes to the fibril size control and orientation of the tissue (Robins 2007). Collagen fibril formation occurs prior to stabilization of the newly-formed fibrils (type I, II, III, V and XI) and those types are crosslinked through reactions between lysine or hydroxylysine aldehydes formed by lysyl oxidase (Eyre et al. 1984). Two aldehydes in close proximity can go through a condensation reaction, the result of which is that a divalent crosslink is formed. These divalent crosslinks consist of Schiff base double bonds and only join two collagen molecules together. Notably, these divalent crosslinks are reducible by heat (Du and McCormick 2009) and can react with other allysines or hydroxyallysines to form trivalent crosslinks that bind three collagen molecules (Lepetit 2008), the formation of which further increases the strength of collagen fibrils.

This mechanism ensures that the crosslinking process is progressive, meaning that ultimately non-reducible crosslinks will be formed and divalent crosslinks will be replaced with trivalent crosslinks, which include lysyl- and hydroxylysyl-pyridinoline and Ehrlich's chromogen (Kuypers et al. 1994). Horgan et al. (1991) found that the trivalent crosslinks were heat stable and their appearance in muscle was correlated with the thermal stability of collagen. The stability of collagen increases as an animal ages so that external factors do not readily modify the collagenous tissue (Nishimura 2010). Age-related changes in crosslink type and their densities are proportional to the rate of animal growth so that by maturity reducible crosslinks represent the minority of collagen crosslinks and the collagen fiber become resistant to heat and is termed heat-insoluble (Robins et al. 1973). The divalent crosslinks therefore represent an intermediate state of crosslinks as by maturity trivalent crosslinks will be formed and will predominate (Bailey et al. 1974). Jackson and Bentley (1960) and Verzár (1963) noted that collagen trivalent crosslinks increase within the collagen macromolecule as the animal ages. Those mature crosslinks from mature animals are located in type I collagen, but were initially in type III collagen (Kovanen and Suominen 1989). Measuring pyridinoline crosslinks may be a predictor of meat toughness (Lepetit 2007) although this may vary with muscle because of contradictory correlations with the level of toughness (Young et al. 1994). These studies indicate that

age is the main factor contributing to the transition from divalent to trivalent crosslinks in muscle hence the importance of reducing the slaughter age of animals to ensure a high-quality product.

1.7 Post mortem ageing

Post mortem ageing is the process of storing of fresh beef at refrigerated temperature (-1 to 4 °C) immediately post mortem. The purpose of post mortem ageing is to improve the eating quality of beef, particularly its tenderness (Love 1994). Proteolytic enzymes are responsible for protein degradation in beef muscle tissue (Goldberg and Dice 1974). Enzymes like calpains and cathepsins, which are active at intramuscular pH values less than physiological pH (6.9 to 7.4) (Kapprell and Goll 1989) breakdown sarcomeric proteins (Robert et al. 1999). Each of these proteolytic enzyme families plays a distinct role in post mortem degradation of muscle, and managing post mortem ageing to gain the desired quality of beef requires an understanding of each of these enzyme families and their functions.

1.7.1 Calpains and calpastatin

Calpains are a family of intracellular proteinases that degrade muscle proteins and regulate the activities of other proteins. There are two types of calpains found in mammalian tissue, the μ-calpains and m-calpains (Sorimachi et al. 1997). The calpains are a calcium-activated family of enzymes and this was discovered when increasing the concentration of Ca^{2+} in muscle *in vitro* removed Z-disks, which are responsible for anchoring the thin filaments to adjacent sarcomeres, without compromising the thick and thin filaments in skeletal muscle myofibrils (Goll et al. 1992). During post mortem ageing, calpains degrade myofibrillar proteins such as desmin, nebulin, and titin in addition to the proteins of the Z-disks, compromising the integrity of the sarcomere structure (Koohmaraie 1994; Lonergan et al. 2010).

During the slaughter process, once an animal has been rendered insensible, killed by exsanguination and the hide or skin removed, the muscle pH and temperature start to decline and the calcium concentration begins to elevate as the Ca^{2+} - Mg^{2+} ATPase transport pump that returns Ca^{2+} to the sarcoplasmic reticulum begins to fail due to lack of adenosine triphosphate (ATP) (Etherington 1984). In live muscle, the cytoplasmic calcium concentration is 1μM and in the post-mortem state it increases to 100μM (Koohmaraie 1992). According to Yoshimura et al. (1983), the Ca2+ concentration at which μ-calpain is completely active is 10μM although there is no precise condition for the best activity of calpains as some authors (Zeece et

al. 1986) agreed that calpains are active at pH values from 6.5 to 7.5. Calpain activity has been shown to vary with breed, as has the activity and concentration of its inhibitor calpastatin, with the Angus breed having the most favorable polymorphisms and the most tender beef (Martins et al., 2017; Leal-Gutiérrez et al., 2018). There are two genes related to the breakdown of myofibrillar proteins, with the gene that codes for calpain (*CAPN*) located on BTA29 and the one that codes for the calpastatin (*CAST*) located on BTA7. Expression of the latter gene regulates the process of proteolysis in meat with increased expression related to increased tenderness (Wheeler and Koohmaraie 1994). Single nucleotide polymorphisms (SNPs) have been identified for *CAPN* and *CAST*, and SNPs identified that are associated with increased meat tenderness scores (White et al. 2005; Barendse et al. 2007b) that have been subsequently commercialized (Plastow and Bruce, 2014). Lee et al. (2014) found that the SNP *CAPN1*:c.1589G > was associated with increased meat tenderness and juiciness and that *CAPN1*:c.1589G > A (Val530Ile) also was associated with increased meat tenderness score. Leal-Gutiérrez et al. (2018) found 16 μ-calpain SNPs and 28 calpastatin SNPs related to meat tenderness in a study of Brahman-Angus cattle with cattle that had 82% or more Angus genetics producing the most tender beef. Also, in the same study, calpastatin SNPs located at 7-98566391 and 7-98581038 were specifically related to an increase in beef tenderness. These results are notable in that they indicate that SNPs can be used to increase beef tenderness through individual animal and breed selection.

1.7.3 Cathepsins

Cathepsins are a family of proteolytic enzymes that are found in lysosomes as they are most active at pH values between 5.5 – 6.5 (Goll et al. 1983; Etherington 1984). Despite being confined in lysosomes, cathepsins may be released post mortem when intramuscular pH declines during post-mortem glycolysis, which could compromise the integrity of the walls of the lysosomal organelles and allow the release of the lysosomal proteinases (Etherington 1984). Ouali et al. (1987) suggested that cathepsins B and L would be most influential during post-mortem ageing as they target myosin and actin (Jiang et al. 1994), while cathepsin L degrades troponin T, titin and nebulin and slowly disintegrates tropomyosin. There was controversy about the effect on meat tenderness and lysosomal rupture but the conclusion was that aged meat showed evidence of cathepsin activity by which proteins were affected (Hopkins and Geesink 2009). Cathepsins in beef may be involved in tenderization processes late in post mortem ageing when low muscle

pH conditions prevail as they are enzymes that are most active at acid pH values (Christensen et al. 2004). However, Caballero et al. (2007) found that the activity of cathepsins plays a key role during prolonged beef ageing as cathepsins B and L were most active late post mortem between days 14 and 21 post mortem.

1.7.4 Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are responsible for extracellular matrix component degradation, turnover and remodeling and require zinc as a cofactor (Christensen and Purslow 2016). The enzymes are activated as Pro-MMP outside the cell by other MMPs that are serine proteinases. MMPs are degraded and inhibited by tissue inhibitors of matrix metalloproteinases (TIMPs) (Mannello and Gazzanelli 2001) and, in some cases, TIMPs can activate gelatinases such as MMP-2 and MMP-9, which degrade collagen that is not yet crosslinked (Christensen and Purslow 2016). In bovine skeletal muscle and connective tissue, (Balcerzak et al. 2001) found 15 MMPs and 3 TIMPS.

The expression of MMP enzymes in beef cattle varies due to animal breed, age, sex, feed level, level of exercise activity and muscle fiber type in muscle tissue (Purslow 2002). Connective tissues appear to be different between various muscles, as Archile-Contreras et al. (2010) showed using 3 different muscles in the same cattle that the activity of MMPs was different between muscles. Other studies have showed an increase in expression of MMP-2 in skeletal muscle that has a high percentage of fast fibers in contrast to the levels of MMP-2 and MMP-9 being undetectable in skeletal muscle fibres composed of highly oxidative slow fibres (Carmeli et al. 2005).

1.7.5 Effect of post-mortem ageing on carcass and meat quality

In the study of Dransfield et al. (1992), the authors stated that 68% of the variation in shear force could be due to the activity of μ-calpains. On the other hand, McDonagh et al. (1999) indicated that calpain and calpastatin were linked to myofibrillar fragmentation index, a common estimation of muscle fragility. Lysosomal enzymes may also work in synergy with the calcium-dependent calpain proteinases (Ouali et al. 1983). This activity coincides with decreases in measurement related to the force of cutting meat, specifically Warner-Bratzler shear force (Franco et al. 2009), and increases in tenderness ratings by trained consumer in sensory panels (Campo et al. 1999). Other factors involved in the process of post mortem ageing include intramuscular pH and the rate of temperature decline during rigor development (Bruce and Ball 1990), which can vary with carcass size due to breed, fat cover due to animal sex (McCormick 1994;

Nishimura 2010), muscle fibre type and collagen content (Dransfield et al. 1981; Ouali et al. 1983), or if electrical stimulation is used during the slaughter process (Marsh et al. 1987).

Smith et al. (1978) tested the effect of the length of post-mortem ageing on palatability and tenderness of beef and showed that the maximum tenderization response was achieved by eleven days of ageing. Franco et al. (2009) showed that shear force decreased relative to non-aged product with 7 and 14 days of ageing. Similar results were found by Monsón et al. (2004) who found that 14 days ageing post-mortem produced the minimum mean shear force value. These results contrasted with those of Campo et al. (1999), who needed 14 days to increase sensory tenderness. Sensory tenderness and Warner-Bratzler shear force estimations of toughness are not exactly comparable, however, as correlations between sensory and mechanical measurements of meat toughness are often poor and can vary between muscles (Chambaz et al. 2003; Calkins and Sullivan 2007). The literature underscores that the effect of post-mortem ageing is a complex process of myofibrillar and connective tissue degradation by endogenous proteolytic enzymes that lead to tenderization of meat.

1.7.6 Post mortem ageing and collagen solubility

Variation in collagen solubility arises due to changes in the ratio of divalent and trivalent crosslink concentrations as a result of the increase in the age of the animal (Bailey and Light 1989). Using one-fourth strength Ringer's solution, Hill (1966) showed that collagen heat solubility decreased with the chronological age of the animal. Purslow (2004) proposed that two pools of collagen molecules exist with one pool accounting for the strength of intramuscular connective tissue (IMCT) due to it containing trivalent crosslinks that are resistant to the process of cooking and ageing and a second pool that is easily dissolved by heat. Studies conducted by Palka (1999) in which semitendinosus (ST) muscle slices were heated at several temperatures, showed no changes in collagen solubility at 50˚C but collagen was highly soluble at 70˚C. In a further study, this researcher indicated that collagen solubility increased with ageing post-mortem in bovine semitendinosus muscle and this increase coincided with a decrease in WBSF (Palka 2003). These studies indicated that collagen solubility can be related to beef toughness as measured by WBSF, and that

increases in collagen solubility with post mortem ageing can contribute to a reduction in the background and overall toughness of beef.

1.7.7 Effect of post mortem ageing on meat quality measurements

In their study Ertbjerg and Puolanne (2017) mentioned that sarcomeres are the smallest contractile units in muscle and they contract and relax to produce movement and heat in cattle. The length between Z lines is defined as the sarcomere length and this length depends on the state of contraction, which averages between1.5 and 2 μm (Warris 2000). The production of short sarcomeres is due to the lack of relaxation of the muscle and the consequence of this is the production of tough meat (Marsh and Leet 1966). The longer the refrigerated storage time post mortem the more tender the meat will be (Wheeler and Koohmaraie 1994). Sarcomere length can be measured directly by examining the myofibers under a microscope (Voyle 1971). Davey et al. (1967) demonstrated that 20% of shortening in excised ox muscle does not increase beef toughness but if this contraction increased to 40% then the shear force will increase. The contraction of sarcomeres can be due to cold shortening, a condition caused by muscle that still contains ATP being chilled below 10 °C (Locker and Hagyard 1963). Locker and Hagyard (1963) also found that muscle shortened very quickly at 0 ˚C, but exhibited minimal shortening at temperatures between 14-19 ˚C. To avoid cold shortening, muscle must be either post-rigor, the temperature has to be more than 10 ˚C at rigor, or the ATP concentration must be low (Pearson and Young 2012). The key risk factor for cold shortening is that excess Ca^{+2} is present in the sarcoplasmic reticulum and the muscle is unable to sequester it (Kanda et al. 1977).

Thaw shortening is another quality issue driven by the contraction of sarcomeres and this is caused by high concentrations of cytoplasmic Ca^{2+} occurring after pre-rigor muscle is frozen before its ATP is exhausted and then thawed because the sarcoplasmic reticulum is damaged by ice crystals formed during freezing. The muscle cannot sequester the Ca^{2+}, and the availability of ATP results in a massive contraction (Bendall 1973). The best way to eliminate this quality issue is to implement the use of electrical stimulation to hasten the onset of rigor mortis through the exhaustion of ATP (Gilbert et al. 1977). Due to the shortening of the sarcomere, cellular water is released and the water holding capacity of the meat is compromised.

The term water holding capacity (WHC) is defined as the amount of water that meat holds either partially or all on its own (Hamm 1986). Approximately 75% of meat is water, with 10% of this water attached to the

muscle proteins and 5 – 10% held in the extracellular space contained between the thick and thin filament (Warris 2000). Water holding capacity can be affected by physiological factors, and conditions associated with animal rearing, and slaughter and processing of a carcass (Enfält et al. 1997). The decline of pH influences drip loss and other problems related to protein denaturation (Bertram et al. 2002), and using growth-promoting supplements like ß-adrenergic agonists can have a negative effect on water holding capacity due to lower fat deposition and increased protein denaturation (den Hertog-Meischke et al. 1997). Girard et al. (2012) found increased water holding capacity with the use of growth promoters in the *gluteus medius* (GM) and ST and a shift from slow oxidative to fast glycolytic muscle fibres in the GM muscle.

The value of beef meat is driven by its palatability and palatability of beef is characterized by its tenderness, juiciness, and flavour, and the consumer tends to pay additional money if the product is tender (Miller et al. 1996; Boleman et al. 1997). The method most used to measure meat toughness is the Warner-Bratzler shear force, a method proposed by K.F. Warner and modified by L.J. Bratzler in the 1930s. The meat sample is cut with a 1 mm thick metal blade perpendicular to the muscle fibre direction that is triangular-shaped and has a hole in the blade. The force required to move the blade and shear the sample is then measured. A graph displaying a curve with two peaks is produced, with the first peak denoting the first yield and the second one the point at which the sample is completely sheared. The area under the curve shows the total work in shearing the sample where the y-axis represents the amount of force required and the x-axis represents the progress of time during the shear (Warris 2000). Several factors contribute to the tenderness/toughness of beef including the amount of marbling, post-mortem proteolysis, connective tissue amount and crosslinking, and the state of the contractile myofibrillar proteins (Belew et al. 2003). Keith et al. (1985) found that the most tender muscles among the thirteen in their study were the *M. psoas major, M. infraspinatus*, M. longissimus dorsi loin and M. longissimus dorsi rib. The muscles ranked as tough were M. pectoral, M. biceps femoris, M. supraspinatus and M. adductor. Torrescano et al. (2003) found that shear force values of *M. gluteus medius and* M. *semimembranosus were c*lassified as muscles with medium toughness. These results emphasize why it is important to understand the meat quality characteristics of each muscle from the beef carcass so that the meat industry can use that knowledge to full advantage through the development and launch of new products to the market or to work toward adding value to muscles that are classified as tough.

1.8 Genomics on meat quality

Because consumers are constantly seeking beef of high quality the beef industry is investing in research with the objective to identify indicators of quality. Beef quality includes tenderness, flavor, color, juiciness among others (Hocquette et al. 2012). The material genetic encoded as DNA in an organism is known as the genome and it includes both genes and non-coding sequences (Lee 2013). Now that the whole genome sequence of the bovine is known (Elsik et al. 2009), identification of genes involved in specific quality traits of economic importance for the producer can be explored (Mullen et al. 2009).

Single nucleotide polymorphisms (SNPs) have been discovered that are associated with specific changes in the function of fat metabolism and deposition, growth rate, and beef quality (Mullen et al. 2009) (Table 1-3). Some of these genes are now able to be measured using commercial tests for polymorphisms that are related to a specific meat quality phenotype (Mullen et al. 2009). For example, serpin peptidase inhibitor, clade E, member 1 (*SERPINE1*) and pentraxin 3, long (*PTX3*), which are genes associated with obesity, were more highly expressed in crossbred Charolais x Red Angus (CHRA) which had higher back fat thickness than Hereford x Aberdeen Angus (HEAN) at slaughter. These results suggest that CHRA may have been at the point where subcutaneous fat accretion was being upregulated, while in the HEAN carcasses it may have been less of a priority (Jin et al. 2012). Huang et al. (2017) in their study of subcutaneous adipose tissue of Wagyu and Holstein cattle, analyzed 662 genes and found that some were highly related to adipogenesis and lipid metabolism such as *PPARγ,* (peroxisome proliferator activated receptor gamma), *PLIN2* (perilipin 2), and *ELOVL6* (fatty acid elongase 6), while other genes like *EGR1* (early growth response 1), *FOS* (fos proto-Oncogene), *AP-1* (transcription factor subunit), *SERPINE1, AGT* (angiotensinogen), and *MMP2* (matrix metallopeptidase 2) may affect adipocytes.

Table 1-3 Single nucleotide polymorphisms (SNPs) in candidate genes influencing beef quality (compiled from Mullen et al. 2009)

Gene	Acronym	Gene function	Biological interest	Reference
Thyroglobulin	*TG*	Affects lipid metabolism	Marbling, IMF	Barendse 1999

Diacylglycerol	*DGAT*	Triglyceride synthesis	Marbling, IMF	Grisart et al. 2002
O-acetyltranferase Leptin	*Leptin*	Associated with growth and fat deposition	Marbling, IMF	Buchanan et al. 2002
Retinoic acid receptor-related orphan receptor C	*RORC*	Member of the steroid and thyroid hormone receptor superfamily	Fatness traits	Barendse et al. 2007a
Bovine Growth Hormone gene	*GH*	Associated with growth	Tenderness and milk yield	Zhang et al. 1993
Growth Hormone Receptor	*GHR*	Interacts with Growth Hormone	Growth traits, IMF	Di Stasio et al. 2005
Fatty Acid Syntahse	*FASN*	Regulates de novo biosynthesis of long-chain FA	Fatty acid composition	Morris et al. 2007
Stearoyl-coA Desaturase	*SCD*	Converts saturated fatty acids to mono-unsaturated fatty acids	Fatty acid composition	Taniguchi et al. 2004
Calpain I (mu/I)	*CAPN1*	Proteolytic activity	Tenderness score, shear force, juiciness, flavour intensity	Page et al. 2002
Myostatin	*Myostatin*	Mutation of gene causes double muscling	Muscle growth	Grobet et al. 1998
Calpastatin	*CAST*	Calpain inhibitor activity	Tenderness, shear force	Barendse 2002; Schenkel et al. 2006

Adipose Fatty Acid Binding Protein Gene	*AFABP4* AFABP4	Fatty acid transport	IMF	Michal et al. 2006

1.8.1 Transcriptomics investigations into meat quality

Transcriptomics refers to the study of all sets of mRNA molecules (transcripts) from a population of cells, and with this technique it is possible to evaluate gene expression of a tissue (Mullen et al. 2009). Gene expression studies can range from the study of a single gene to analysis of complete gene expression patterns and profiles of many genes in a livestock system (Campbell et al. 2001). Genes have been studied in relation to the tenderness, juiciness, flavour and WHC of beef (Hocquette et al. 2009). The use of real time polymerase chain reaction (RT-PCR) employs amplification kinetics to compare the level of the expression of specific or multiple genes against one housekeeping gene (Schefe et al. 2006).

The application of transcriptomics to beef quality appears to be limited. Gene expression has been investigated in the muscles of different breeds (Benkeblia 2012). Lehnert et al. (2007) examined the expression of *FSTL1* (follistatin-like 1) and *IGFBP5* (insulin-like growth factor binding protein 5), which have been implicated in muscle cell growth and cell differentiation and related to myogenesis regulation, in developing cattle muscle in different stage of birth. This review found limited literature describing variation in the expression of genes related to collagen biosynthesis and degradation with breed and residual feed intake. By Identifying genes that vary in their expression with phenotypical differences, we can try to understand the genes and molecules involved in the processes of collagen biosynthesis and fibrogenesis associated with growth and adipogenesis, and degradation of the ECM that can be related to toughness and marbling in meat (Harper 1999).

1.9 Hypotheses and objectives

This literature review demonstrates that the impact of RFI on beef quality is equivocal and its interaction if any with breed is not clear. The review also revealed that limited research exists that relates gene expression to intramuscular collagen and whether expression of genes involved in collagen synthesis and

degradation are affected by RFI and breed. Understanding these relationships could lead to significant insight into the biological mechanisms governing collagen solubility and its contribution to beef toughness. The hypotheses tested in this thesis therefore were:

1.- That breed and RFI affect meat quality and collagen characteristics of the bovine m. gluteus medius;

2.- The relative expression of genes involved in collagen biosynthesis and degradation In the bovine m. gluteus medius are correlated with total collagen, collagen heat solubility and meat quality of that muscle.

The objectives of this thesis were therefore to test these hypotheses by investigating the effect of high and low RFI status in steers from three breeds on beef quality and collagen characteristics of the m. gluteus medius, and characterizing the expression of 38 genes involved in collagen synthesis and degradation.

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Chapter 2
The influence of breed and residual feed intake status on meat quality and intramuscular collagen properties of bovine m. *gluteus medius*

2.1 Introduction

Meat producers are seeking constantly to improve meat quality traits in order to be commercially profitable. One way to obtain additional profits in beef production is by selecting animals that are the most efficient in terms of the digestion and use of feed (Arthur et al. 2004). Selecting specific breeds for weight gain is one strategy for increasing animal efficiency through increased growth rate. Barton et al. (2006) compared Aberdeen Angus, Charolais, Simmental and Hereford cattle and found differences in live weight, with Charolais and Simmental cattle gaining weight faster than Aberdeen Angus. These results confirmed that later maturing breeds like Charolais and Simmental tended to gain more weight at the same age as early maturing breeds.

Selecting cattle for low residual feed intake (RFI) is another strategy for decreasing beef production costs through reduction of feed costs (Arthur et al. 2004). There appears to be an indirect effect of selecting cattle for low RFI, including decreased carcass fat and marbling, which could affect meat quality (Basarab et al. 2003). Within meat quality traits, tenderness is the most important trait for the beef industry (Miller et al. 2001), as consumers are willing to pay between 1.7 and 2.9 times additional money for beef that is tender and juicy (Lyford et al. 2010). There is still no conclusive result in the use of RFI, but authors like Lorenzen et al. (1993), Pesonen et al. (2012) and Ba et al. (2013) found differences in fat between different breeds, with the last authors finding that cooking loss was affected by RFI in the Angus and Hanwoo breeds. These results indicate that RFI may exert an effect on the quality of beef by changing beef moisture and fat.

Different factors influence meat tenderness, with degradation in the structure of myofibrillar proteins during post-mortem ageing contributing significantly to increased tenderness (Bouton et al. 1981). Sarcomere length (Bouton et al. 1974), muscle fiber size and type (Bouton et al. 1978), and breed (Sañudo et al. 2004) can also influence the quality of beef and cause variation in it. Additionally the biological influence of connective tissue is important as well, as an increased amount of intramuscular collagen is associated with

increased beef toughness (Ngapo et al. 2002). The strength of collagen affects its heat solubility, with heat solubility decreasing with animal age (Hill 1966), due to the complexity of its crosslinks increasing with animal age (Bailey and Light 1989; Nishimura et al. 1996). The complexity of collagen crosslinks increases as divalent crosslinks condense into trivalent crosslinks (Eyre and Wu 2005), and this is associated with increased meat toughness.

Collagen characteristics are particularly pertinent to bovine muscles in which toughness increases substantially with age (Hill 1966). Girard et al. (2012) found that the toughness of the m. *gluteus medius* as estimated by Warner-Bratzler shear force increased with beef steer age, suggesting that cattle age differences may contribute to the perception of tenderness variability by beef consumers for beef cuts from this muscle and reduce its marketability or value. Although the effect of breed and RFI on beef toughness has been examined, there is limited literature on the effects of breed and RFI on the intramuscular collagen characteristics of beef. When studying the collagen characteristics, texture and sensory variables of 10 different Spanish and French beef cattle breeds, Panea et al. (2018) found a significant effect of breed on all collagen and texture characteristics, with two breeds (Asturiana de las Montañas and Gasconne) showing significantly smaller total collagen values compared to the other breeds. Additionally, a large variation in the collagen solubility values was found within breeds for the same study (Panea et al. 2018). In their study Girard et al. (2012) found no effect of breed in total collagen and collagen solubility percentage within breed crosses Charolais-Red Angus and Herford -Aberdeen Angus. For the effect of RFI on collagen, Zorzi et al. (2013) found that the selection for low RFI increased soluble collagen in Nellore bulls, but whether these results persist in other cattle breeds, particularly those of the *Bos taurus* sub-species is unknown. The objective of this study then was to investigate the effects of breed, RFI and their interaction on intramuscular collagen characteristics of the m. *gluteus medius*, testing the hypotheses that RFI reduces intramuscular fat content without affecting beef toughness or collagen characteristics; that breed differences exist for meat quality and collagen characteristics; and that RFI and breed do not interact to affect beef quality.

2.2 Materials and methods

2.2.1 Animals

Muscles from the carcasses of cross-bred Angus (Kinsella Composite) (n=24), purebred Angus (n=23 as one died before slaughter) and Charolais (n=24) steers were used to test the hypotheses examined in this study. This study was reviewed and approved by an Animal Ethics Committee at the University of Alberta (Animal Use Protocol 00000777). Angus crossbred steers were produced by crossing Angus bulls with a hybrid dam line (Kinsella Composite), with the hybrid dam line consisting of approximately 33% Angus, 33% Charolais and 20% Galloway, and the remainder consisting of other beef breeds as described in detail by Goonewardene et al. (2003) and Nkrumah et al. (2007). All calves were born in April or May of 2013, on the Roy Berg Kinsella Research Ranch, University of Alberta, and remained there under similar management conditions for the duration of the study.

2.2.2 Cattle management and RFI determination

Cattle were managed as described by Jiang et al. (2012). Shortly after birth, the calves were uniquely identified by ear tags and castrated by banding. Steer calves remained with their dams on pasture until weaned at approximately 190 days of age, after which they were gradually alimented onto a background diet of 65.1% oats and 34.9% hay. Steers received this background diet until approximately 11 months of age. Steers were then gradually alimented over approximately 3 weeks onto a finishing diet of 75% barley grain, 20% barley silage, and 5% pellet supplement that included Rumensin^{TM} (as fed, 76.5% dry matter; 14.7 % crude protein, 18.3% acid detergent fibre, 32% neutral detergent fibre, 1.2% calcium, 0.45% phosphorus, and 70% total digestible nutrients on a dry matter basis; 0.24 ppm Mg, 0.93 ppm K, 0.28 ppm Na, 604 ppm Fe, 128 ppm Mn, 182 ppm Zn, and 29.4 ppm Cu). Steers received this finishing diet during and after RFI testing.

For RFI testing, steers were placed by genetic group into feedlot pens (4 for Angus crossbreds, 1 for purebred Angus and 1 for purebred Charolais) fitted with the GrowSafe system (GrowSafe Systems Inc., Airdrie, AB, Canada), and individual feed intake was monitored daily during the finishing period. Angus and Charolais steers were tested for RFI from June to August and Angus crossbred steers from April to June due to anticipated finishing/slaughter date and limitations in GrowSafe capacity and availability. Testing for RFI was conducted at different times to accommodate differences in physiological age (Loyd et al. 2011) between the breeds. The use of the GrowSafe feeding system was described by Basarab et al. (2003).

Body weight, ultrasound and rib eye area measurements were described by Mao et al. (2013). Briefly, body weight (BW) of the steers was measured twice at the start of the feedlot test, once every 14 days, and twice at the end of the test. Ultrasound measurement of back fat thickness and *longissimus thoracis* (LT, rib eye) area at the 12^{th} – 13^{th} rib was performed at the end of the feedlot test period using an Aloka 500V diagnostic real-time ultrasound with a 17 cm 3.5 MHz linear array transducer (Overseas Monitor Corporation Ltd., Richmond, BC, Canada).

Individual steer RFI values were calculated as the difference between a steer's actual and predicted dry matter intake (DMI), with the equation: RFI = DMI actual – DMI predicted. RFI values were calculated for all calves within each breed. DMI predicted was calculated using animal average daily gain (ADG) and metabolic BW (MWT) calculated as midpoint $BW^{0.75}$, where midpoint BW was the sum of initial BW and ADG multiplied by half of the days on test (DOT) (Equation 1). Residual feed intake was also adjusted for back fat thickness (RFIf), where RFIf = DMI actual – DMI predicted based on ADG, MWT and final ultrasound back fat (FUFAT) measured at the end of the test (Equation 2), to negate effects on RFI due to differences in physiological age (Basarab et al. 2011). The models used to predict expected DMI and expected DMI adjusted for ultrasound back fat thickness were described by Mao et al. (2013) and are presented respectively here:

$$Y_i = \beta_0 + \beta_1 ADG_i + \beta_2 MWT_i + e_i \quad \text{(Equation 1)}$$

$$Y_i = \beta_0 + \beta_1 ADG_i + \beta_2 MWT_i + \beta 3 FUFAT_i + e_i \quad \text{(Equation 2)}$$

Where β_0 is the intercept; β_1 β_2 and $\beta 3$ are the coefficients on ADG, MWT and FUFAT, respectively; e_i is the residual. The DMI adjusted for ultrasound back fat thickness in model (2) was used to calculate RFIf.

From RFI test values, steers were classified as negative (low) RFI and positive (high) RFI. Twenty-four steers from each breed (one Angus steer died prior to slaughter) were selected based on RFI and balanced for body weight, with 12 steers having an RFI less than or equal to -0.13 kg (negative RFI) from the mean RFI and 12 having an RFI greater than 0.13 from the mean (positive RFI) within each breed.

Steers were finished to a back fat of between 8 and 10 mm estimated using ultrasound according to normal industry practice; therefore, breeds were finished and slaughtered at different times to accommodate the differences in physiological age and were slaughtered within approximately one month of completing RFI

testing. Within each breed, steers were slaughtered over two days with 12 steers killed each day from July to September in 2015, with the exception of Angus steers, which had 8 killed over 3 slaughter days. On each slaughter day, 6 steers from each RFI treatment (high, low) were processed, and for Angus 4 steers from each RFI (high, low) were processed. Mean kill ages were 452, 533, and 511 days for Angus crossbred, Angus, and Charolais, steers, respectively. Each animal was finished to greater than 2 mm back fat prior to slaughter. The animals were received at the abattoir and rested with *ad libitum* access to water for approximately 2 h prior to slaughter. Animals were slaughtered at the Meat Research Laboratory abattoir at Agriculture and Agri-Food Canada, Lacombe, Alberta, a federally inspected abattoir. Both animals and carcasses were inspected by trained personnel from the Canadian Food Inspection Agency and each carcass was graded by trained personnel.

2.3 Carcass and sample management

Carcasses were chilled for approximately 72 h at 1 °C in a chiller with a wind speed of 0.5 m^2/ second. After 72 h, carcasses were ribbed between the 12^{th} and 13^{th} ribs and the cut face of the LT exposed to atmospheric oxygen a minimum of 10 minutes as per Canadian beef grading legislation (Canada Gazette, 2019). Each carcass was graded (Canada Gazette, 2019) and evaluated by personnel certified by the Canadian Beef Grading Agency. Following grading, whole GM muscles were removed from the right sides of the carcasses and fabricated into 2.5 cm steaks for the analysis of meat quality traits.

During fabrication of the GM, steaks were removed cranial to caudal with the first steak used for drip loss and colour, the second for proximate analyses, the third and fourth for Warner-Bratzler shear force at days 3 and 13 post mortem, respectively, the fifth and sixth for collagen analysis on day 3 and 13 post-mortem in this study wet aging (vacuum aging) was performed as described by Jiang et al. (2010). Steaks were removed in differing orders to ensure that steaks used for the collagen and WBSF analyses were from the widest portions of the muscle where muscle fibre direction was as homogeneous as possible. All steaks were packaged under vacuum in polypropylene bags at about 2 °C until the completion of the prescribed post-mortem ageing period and then frozen at -20 °C, with the exception of those used to measure drip loss.

2.4 Meat quality

2.4.1 Temperature, pH and purge

Prior to fabrication of each muscle into steaks, intramuscular temperature and pH were recorded using a Fisher Scientific Accumet AP72 pH meter (Fisher Scientific, Mississauga, ON, Canada) equipped with an Orion Ingold electrode (Udorf, Switzerland) and fitted with a temperature probe. (Type T copper-constantan, 10 cm in length, AllTemp Sensors Inc., Edmonton, AB) Three readings for pH and temperature were performed and the mean of each used for statistical analysis. The weight of the packaged steak was recorded before and after it was removed from the packaging, with excess moisture drained from the package and the packaging itself weighed. Purge loss was determined by subtracting the weight of the steak and its package from the package containing the steak prior to being opened, and it was expressed as milligrams water loss per gram of muscle.

2.4.2 Proximate analysis

For proximate analysis, a 2.5 cm steak was trimmed of epimysium and surface fat, ground (Robot Coupe Blixir BX3; Robot Coupe USA Inc., Ridgeland, MS, USA) and the ground sample analyzed for protein using a CEM Rapid Analyzer System (Sprint Protein Analyzer Model 558000, CEM Corporation, Matthews, NC, USA), for moisture (Smart Turbo Moisture Analyzer Model 907990, CEM Corporation, Matthews, NC, USA) and fat (Smart Trac Fat Analyzer Model 907955, CEM Corporation, Matthews, NC, USA) contents (Method 2008.06) (Leffler et al. 2008). Compositional contents were expressed as a percentage of sample weight.

2.4.3 Sarcomere length

Sarcomere length was measured following the method used by Aalhus et al. (1999). Two grams of muscle free of connective tissue and fat were minced with scissors, and then were mixed with 0.25 M sucrose/0.02M EGTA in a 50 mL centrifuge tube. The mixture was homogenized for 10 seconds at 8064 x g (Polytron Homogenizer PT3100 and a 2 cm generator (Brinkmann Instruments Inc. Mississauga, ON, Canada). One drop of homogenized sample was covered with a cover slip to prevent dehydration and a phase contrast microscope (Axioscope, Zeiss, West Germany) fitted with a Sony DXC 930 Colour Video Camera (Sony Corporation, Japan) was used to capture 12 images of sarcomeres per steak sample. Sarcomere lengths were averaged and quantified in μm for each steak and the mean sarcomere length was calculated from the best ten images for statistical analysis.

2.4.4 Drip loss

Drip loss was estimated using the method described by Aldai et al. (2010). Briefly, the steak harvested at 72 h post mortem was weighed and then placed on a polystyrene over-wrapped tray with a Dri-Loc® pad (Cryovac Inc., Charlotte, North Carolina) for 4 days at 2 °C, and then weighed again. Drip loss was the difference between the initial and final weights of the steak expressed in milligrams water lost/gram of muscle.

2.4.5 Cooking loss and cooking time

Each steak was weighed and a thermometer probe (Type T copper-constantan, 10 cm in length, AllTemp sensors Inc., Edmonton, AB) placed in the geometric center of the steak cross-section. Steaks were heated at approximately 210˚C (Garland Grill ED30B; Condon Barr Food Equipment Ltd., Edmonton, AB), with the internal temperature recorded every 30 seconds. When the internal temperature reached 35.5˚C the steak was turned and cooked until an internal temperature of 71˚C (monitored with a Hewlett Packard HP34970A Data Logger; Hewlett Packard Co., Boise ID). At 71˚C was reached. Cooked steaks were placed in polyethylene bags and then cooled in an ice bath to prevent overcooking. Steaks were then placed in a refrigerator at 4˚C overnight. Cooking time was recorded and was defined as the time in seconds required to cook 1 g of raw steak. Cooking loss was recorded as well, with steaks dried with filter paper prior to the weight of each steak being recorded. Cooking loss was expressed in mg of water lost per g of raw steak.

2.4.6 Warner-Bratzler shear force (WBSF)

The day following cooking, six 1.9 cm cores per steak of were removed from cooked steaks parallel to muscle fibers. Samples were analyzed using a Texture Analyser (Model TA.XT plus, Texture Technologies Corp, New York) equipped with a Warner-Bratzler shear blade, measuring peak shear force (SF) at a crosshead speed of 200 mm/min. Peak shear force of the six cores per muscle were recorded in kg and the average and standard deviation were calculated and used for statistical analysis.

2.5 Collagen characteristics

2.5.1 Intramuscular connective tissue (IMCT) isolation

Steaks aged 3 and 13 days were removed from frozen storage and thawed at about 4˚C for 24 h. Thawed steaks were trimmed of epimysium and subcutaneous fat, then cubed into approximately 2 cm^3 pieces for

connective tissue isolation. The cubes were mixed by hand to homogeneity and then weighed and the weight recorded. Connective tissue was isolated from about 100 g of the chopped cubes. The cubes were blended with 5 volumes (w/v) of deionized water for 10 s at low speed and then 10 s at high speed using a laboratory blender (Waring, Fisher Scientific, Mississauga, ON). The homogenate was then filtered through a stainless-steel sieve (pore size $1mm^2$) and the residue blended and filtered again twice. The remaining residue was deemed intramuscular connective tissue (IMCT), and it was dried with Whatman No. 4 filter paper (Fisher Scientific, Mississauga, Ontario), weighed and then frozen at -70˚C until lyophilized. Lyophilized IMCT was considered perimysium (Kuypers et al. 1994) and was stored frozen at -20˚C until further use. Following lyophilization, the perimysium was covered and protected from light as the collagen crosslink pyridinoline is sensitive to ultraviolet light (Fujimoto and Moriguchi 1978).

2.5.2 Pyridinoline (PYR) quantification

For the quantification of PYR in IMCT, about 0.20 g of IMCT was hydrolyzed with 6 mL of 6 M hydrochloric acid at 110˚C for 22h in a dry bath. Prior to hydrolysis, each tube was flushed with nitrogen gas to ensure an anaerobic hydrolysis. Following hydrolysis, hydrolysates were cooled in ice water for 15 min to stop hydrolysis. The hydrolysates were filtered using Whatman No. 4 filter paper (Fisher Scientific, Fischer Scientific, Mississauga, Ontario). The total filtered volume was 8 mL from which a 6 mL aliquot was used for pyridinoline (PYR) quantification and the remaining 2 mL were used for estimation of hydroxyproline concentration for total collagen determination.

Concentration of PYR in the IMCT was measured using the method reported by Robins et al. (1996). The 6 mL from the hydrolysis were evaporated to dryness avoiding exposure to light. Once the sample was evaporated, it was reconstituted with 1.5 mL of 10% (v/v) acetic acid. The PYR fractions were isolated using size exclusion chromatography (Bio-Gel P-2, Bio-Rad, Canada) in gravity columns (Econo-Pac, 20 ml, bed volumes, 1.5 cm x 12 cm column. Bio-Rad. USA). The PYR was further purified using cation exchange chromatography column (P11 phosphocellulose, Whatman International Ltd.) with detection using it fluorescence at λ_{ex} = 295nm, λ_{em} = 400nm (Robins et al. 1996).

Fractions in which PYR was detected were pooled, and 1.0M hydrochloric acid was used to wash the column, and 0.1M hydrochloric acid was used to eliminate any remaining amino acids (Fujimoto and Moriguchi 1978; Yamauchi et al. 1987). The samples were then evaporated to dryness and reconstituted

with 2 mL of 1% aqueous heptafluorobutyric acid (HFBA, Sigma-Aldrich Canada. Ltd), followed by filtering of the sample with a filter (Acrodisc, Pall, Life Science) fitted to a syringe to obtain 1mL for quantification of PYR using reverse-phase high performance liquid chromatography (RP-HPLC). The PYR concentration was quantified by comparing it with a PYR standard (Wako Chemicals, USA). The molecular weight of PYR (429g PYR/mol) was used to report the concentration of PYR in perimysium IMCT. All analyses of PYR were performed in duplicate.

2.5.3 Total collagen quantification

From the PYR hydrolysates, a 2 mL aliquot was used to determine total collagen. The aliquot was evaporated to dryness, reconstituted with 2 mL deionized water, and neutralized to pH 7 with 2N NaOH. The sample was then evaporated and reconstituted to 10 mL with deionized water and stored at -20˚C until use. The assay was performed in duplicate using 0.04 mL of sample and 0.960 mL of deionized water added to each sample. Using the modified version of Bergman and Loxley (1963), the amount of total collagen was estimated by measuring the hydroxyproline (HYP) concentration of the PYR hydrolysates. By diluting 2.5, 5.0, 10.0, 20.0, and 40.0 µg/mL of standard solution of trans-4-hydroxy-L-proline (Sigma-Aldrich, Canada Ltd, Oakville, ON) and measuring the absorbance at 558 nm against a water blank, the hydroxyproline concentration in each sample was determined by linear regression of the sample absorbance against the standard curve. Assuming that hydroxyproline constituted 14% of collagen, the concentration of hydroxyproline in IMCT was multiplied by 7.14 to estimate the collagen concentration of the IMCT (Etherington and Sims 1981). The concentrations of collagen in IMCT were used to quantify PYR concentration on a mol PYR/mol collagen basis as a measure of cross-link density and concentration in muscle.

2.5.4 Collagen solubility

The solubility was determined based on the procedure of Hill (1966), in which 1.00±0.01 g of lyophilized ground muscle was incubated with 20 mL of one-quarter strength Ringer's solution at 77 ˚C. After that, the content of the tubes was centrifugated at 4 ˚C for 10min at 3500g, the supernatant was collected (soluble fraction) and placed in -20 ˚C for further analysis. 1 mL of the soluble fraction was used for hydrolysis with 4 mL of 6 M HCL and 1 mL 12M to get a total volume of 6 mL then the samples were hydrolysed for 20-22

h at 110˚C (Accu-BlockTM Digital Dry bath. Labnet International, Inc., Edison, NJ). Hydroxyproline assay was determined as described in the total collagen section 2.5.3 and this was done for both fractions.

2.6 Statistical analysis

Data from perimysial connective tissue from the GM muscle and the meat quality data were analyzed using a linear mixed model In RStudio (RStudio Server Team 2016). A split plot design was used where breed and RFI and their interaction were tested in the main plot and day of ageing (3 and 13 days post mortem) was considered in the split plot. The experimental unit in the whole plot was the GM muscle and the steak was the experimental unit in the sub-plot. Least square means of each treatment were calculated and compared for difference using Tukey's Honestly Significant Differences test, with differences significant level at $P<0.05$ when the interaction or the main effects were significant at $P<0.05$. Pearson's correlations were performed in R studio, with correlations performed between collagen characteristics and meat quality traits using the package Hmisc (Version 4.0-2), and correlations considered significant at $P<0.01$.

2.7 Results

2.7.1 Meat quality traits

There was a significant interaction between RFI and breed on intramuscular pH ($P<0.05$) (Table 2-1). The interaction was significant due to the changes in direction of the interaction slopes only as there were no significant differences between interaction means (Figure 2.1). There was a significant ($P<0.01$) interaction between breed and RFI for sarcomere length also, with the mean sarcomere length of the GM muscle from high RFI Angus steer carcasses being shorter than that from low RFI Angus steer carcasses and that of the other breeds regardless of RFI status (Figure 2.2). RFI had no effect on mean sarcomere lengths of GM from Charolais or Angus crossbred steers.

There was no effect of breed, RFI or their interaction on intramuscular temperature or purge loss (Table 2-1). Proximate analysis results indicated that there was no breed effect on moisture, fat or protein content of the GM, but fat content in GM from low RFI was lower than that from high RFI steers (Table 2-1). RFI did not affect moisture and protein contents of the GM nor did the interaction (Table 2-1). The GM from Charolais carcasses exhibited a greater drip loss than that of Angus and Angus crossbred steers (Table 2-

1). There was no effect of RFI and no interaction of RFI with breed on drip loss. Cooking loss and cooking time were also unaffected by breed, RFI, ageing or their interactions (Table 2-2).

There was no effect of RFI on mean Warner-Bratzler shear force values, but there was a significant effect of the interaction between breed and ageing (Table 2-2). Within the interaction, ageing 13 days post mortem reduced mean WBSF values of the GM steaks for the Angus and Angus crossbred only, with mean WBSF values for the GM from the Charolais steers already at the same WBSF value at day 3 post mortem as that of the other breeds at day 13 (Figure 2.3). Mean standard deviation of the WBSF cores within steak also decreased with ageing in the Angus and Angus crossbred steers, but was unaffected in GM from the Charolais steer carcasses as the core standard deviation for that breed at day 3 of ageing was already as low as that for the other two breeds at day 13 (Figure 2.4).

2.7.2 Collagen characteristics

There was no effect of breed, RFI or their interaction on the percentage of perimysium in the GM (Table 2-1). The concentration of Ehrlich's chromogen in the perimysium differed between breeds, with perimysium from the GM of Angus crossbred steers having the lowest concentration of Ehrlich's chromogen (Table 2-1). There was no RFI or interaction of breed and RFI effect on Ehrlich's chromogen concentration in the GM, nor was there any significant experimental effects on pyridinoline molar density (mol PYR/mol collagen) or intramuscular concentration (nmol PYR/g raw muscle) (Table 2-1).

Total collagen content of the GM was unaffected by breed and RFI, but was lowest in the steaks used at day 13 than used at day 3 post mortem (Table 2-2). Collagen heat solubility expressed as a percentage of total collagen increased with postmortem ageing in the GM muscles from Angus and Charolais steers only; it did not change in the Angus crossbred which at day 3 post mortem had a collagen solubility value similar to that of the other breeds at day 13 post mortem (Fig 2.5). The GM of steers selected for low RFI also had greater mean collagen solubility than that of steers selected for high RFI (Table 2-2).

2.7.3 Pearson correlations

Pearson correlations between meat quality traits and collagen characteristics are presented in Table 2-3 and correlations were considered significant at $P < 0.01$. Intramuscular pH of the GM was negatively correlated with percentage of perimysium (r=-0.44). Intramuscular temperature at fabrication was negatively correlated with drip loss (r=-0.36) and positively correlated with pyridinoline concentration, collagen

solubility percentage 3dpm and density (r=0.60, r=0.43 and 0.69, respectively). Percentage moisture was negatively correlated to percentage fat (r=-0.85) and percentage protein (r=-0.43). Drip loss was negatively correlated to PYR concentration in muscle (-0.39) and PYR density in collagen (-r= -0.36). Perimysium percentage was positive correlated with EC concentration and PYR density in collagen (r= 0.80 and 0.44 respectively). Pyridinoline concentration in muscle was positively correlated to PYR density in collagen and the collagen solubility at 3 dpm (r=0.79 and 0.44. respectively).

Total collagen at day 13 was positively correlated with collagen solubility at day 13 ($P<0.01$). Cooking loss at day 13 was positively correlated with shear force at day 13 post mortem (r=0.37) and cooking time (r=0.67). Cooking loss at day 3 post mortem was positively correlated with shear force at days 3 and 13 post mortem, cooking time at day 13 post mortem and shear force core standard deviation at day 3 post mortem (r=0.71, 0.39, 0.72, and 0.41, respectively). Shear force at day 3 post mortem was positively correlated with shear force at day 13, cooking time day 3 and shear force core standard deviation (r=0.44, 0.45, 0.76, respectively). Shear force at day 13 post mortem was correlated with core standard deviation 13 post mortem (r=0.57).

2.8 Discussion

The results of this study indicated that RFI provides beef producers with an option for increasing animal efficiency and decreasing feed costs without compromising beef quality as estimated using objective technological measurements such as intramuscular pH, sarcomere length, proximate analysis and Warner-Bratzler shear force. This conclusion agrees with that of Baker et al. (2006) and Fidelis et al. (2017), both of whom found no effect of RFI on beef quality. The results disagree with the results of Herd et al. (2018) and Zorzi et al. (2013) who found that low RFI cattle had and tended to have, respectively, greater beef toughness than high RFI cattle. Selection for low RFI was associated with a reduction in intramuscular fat, as was observed by Baker et al. (2006), Ahola et al. (2011) and Herd and Arthur (2009). Similar results to those related to intramuscular fat and selection for LRFI animals of this chapter were found in pigs selected for low RFI (Prunier et al. 2011). However, the results from this chapter do not agree with those of Fidelis et al. (2017), who found no effect of selection for RFI on marbling in Nellore bulls. They also do not agree with the results of Herd et al. (2018) who found that selection for low RFI increased intramuscular crude fat. That selection for low RFI reduced the amount of marbling in the rib eye muscle suggests that the Canada

quality grade may be reduced and further research is warranted to ensure that selection for low RFI does not decrease quality grade as this may decrease the value of the carcass paid to cattle producers.

The intramuscular pH values in this study were close to that expected normally in beef pH (5.5-5.6) (Pearson and Young 2012) and with all mean pH values less than 5.8, it was unlikely that pH influenced toughness or colour (Silva et al. 1999). Most importantly, there was no influence of RFI on intramuscular pH, which agreed with the results of Zorzi et al. (2013) who compared Nellore bulls bred for high and low RFI and found that there was no significant difference in the pH of the m. longissimus. Also, Hunt et al. (2006) in their study found no differences in intramuscular pH due to RFI. These results suggest that WHC or color will not be altered by variation in intramuscular pH in low RFI animals.

There was a significant interaction between RFI and breed for sarcomere length in this study where high RFI Angus had a shorter mean sarcomere length than low RFI, indicating that the selection of Angus steers for efficiency through use of low RFI had no deleterious effects on sarcomere length. Zorzi et al. (2013) also did not find any significant difference in the sarcomere length of the m. *Longissimus* of Nellore bulls due to RFI.

Selection for low RFI did not affect bag purge, drip loss or collagen cross-link intramuscular concentrations or densities within collagen, regardless of crosslink. It also did not affect total intramuscular collagen, cooking loss, cooking time, and shear force. Selection for RFI did increase collagen solubility, agreeing with the results of Zorzi et al. (2013), suggesting that feed efficient animals may produce beef with reduced background toughness due to the decreased heat stability of their perimysium collagen (Lawrie et al. 2006). Shear force in this thesis was used as a measure of toughness, and was not affected by RFI, suggesting that in the GM soluble collagen did not contribute substantially to the total shear force. This was supported by the lack of a significant correlation between soluble collagen and shear force at days 3 and 13 post mortem.

Collagen solubility also decreased with days of ageing in the Angus and Charolais breeds only, confirming that there are differences in early post mortem collagen solubility between breeds. The effect of ageing on collagen solubility was shown by Palka (2003), who found an increase in collagen solubility with post mortem time that was accompanied by a decrease in shear force, This interaction may be due to the Angus crossbred steers having a larger pool of newly synthesized collagen that was not or was newly incorporated

into existing collagen fibrils than the other genetic groups (Purslow 2018), which can arise from physiological differences between the breeds (Blanco et al. 2013). Interestingly, soluble collagen reached a similar proportion across the breeds by day 13, suggesting that there may be a limit to the amount of collagen that can solubilize post mortem. This suggests that the availability of cleavages sites to matrix metalloproteinases may be a limiting factor to increasing collagen solubility with post mortem ageing, with access to cleavage sites possibly limited by cross-links such as PYR (Perumal et al. 2008).

Results also showed that the amount of collagen in the GM was lower at day 13 than at day 3 post-mortem. This result agrees with that of Modzelewska-Kapituła et al. (2015) who indicated that a decline in total collagen with ageing was due to the degradation and disintegration of collagen fibrils and proteoglycans that stabilize the collagen fibril bonds (Nishimura et al. 1996). Most likely the difference in total collagen content between days 3 and 13 in this thesis is due to a difference in the distribution of collagen as two different steaks were used to estimate total collagen at different times post mortem, and the total collagen is more likely to differ due to location rather than post mortem ageing. Collagen is a resilient protein that resists degradation (Purslow 2014), and complete hydrolysis during the 13 days of ageing is unlikely to occur.

As expected, shear force values decreased with number of days post-mortem, but only in the Angus and Angus crossbred steers. In the Charolais steers, mean shear force at day 3 post mortem was equivalent to the mean shear forces of all other steer genetic groups at day 13, suggesting that proteolysis in the Charolais steer muscle was rapid and complete by day 3 post mortem. This might be explained by calpain activity in the muscles of each breed (Wulf et al. 1996), and genetic variation in the amount of collagen type of fibers and enzymatic activity (Mandell et al. 1997). The reduction in shear force was accompanied by a reduction in mean standard deviation of the cores at day 3 and 13 post-mortem, indicating that as the force required to cut the cooked product decreased, so too did the variability in toughness within the cooked muscle.

Breed also affected drip loss and intramuscular EC concentration. Mean EC concentrations were higher than those observed by Roy et al. (2015) in the same muscle, and why the Kinsella crossbred had a significantly lower mean EC concentration than the Charolais is unknown. The difference in intramuscular EC concentration between breeds might be due to genotypic characteristics of each breed (Hocquette et

al. 2006) or differences in physiological age (Blanco et al. 2013). Why there were differences in drip loss due to breed is not clear, but they may be linked to carcass size, as large carcasses cool more slowly than light carcasses (Smulders et al. 1992), and the combination of low pH and high temperature in early post mortem muscle can lead to increased protein denaturation and increased drip loss (Bruce and Ball 1990). These results support close management of carcass chilling regime so that carcass muscles chill at the same rate regardless of carcass size.

2.9 Conclusion

Results of this chapter indicated that RFI did not interact meaningfully with breed for any of the meat quality measurements and that it increased the soluble portion of collagen, which may indicate a reduction in the background toughness of beef with this selection pressure. Selection for low RFI therefore can be used by beef producers to increase beef production efficiency as it does not appear to adversely affect beef quality or intramuscular collagen characteristics.

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2.11 Tables

Table 2-1 Effect of breed and residual feed intake on meat quality composition of the m. *Gluteus medius* including significant interaction

	Breeds				Residual feed intake			Interaction
Variables	AN[1]	CH[2]	KC[3]	p-value	High	Low	p-value	BreedxRFI
n=	23	24	24	-	35	36	-	-
pH	5.47±0.03	5.48±0.04	5.50±0.04	0.90	5.48±0.02	5.48±0.02	0.68	<0.01
Temp (˚C)	2.27±0.38	1.52±0.47	3.40±0.47	0.07	2.39±0.35	2.40±0.34	0.94	0.66
Purge (mg/g)	6.85±0.76	7.10±0.93	9.58±0.93	0.15	8.29±0.56	7.39±0.56	0.14	0.18
Fat (%)	3.87±0.26	3.80±0.30	3.46±0.30	0.61	3.92[a]±0.19	3.51[b]±0.19	0.04	0.21
Protein (%)	20.88±0.21	21.38±0.23	21.03±0.23	0.38	21.04±0.15	21.17±0.15	0.55	0.98
Moisture (%)	73.33±0.25	72.89±0.29	73.47±0.29	0.43	73.16±0.18	73.30±0.18	0.36	0.42
Drip loss (mg/g)	43.92[b]±1.93	48.85[a]±2.0	42.52[b]±2.0	0.01	45.17±1.76	45.03±1.70	0.93	0.37
Sarcomere length (µm)	1.60±0.03	1.68±0.03	1.71±0.03	0.16	1.67±0.03	1.65±0.03	0.58	<0.01
Perimysium (%)	1.52±0.18	1.55±0.19	1.47±0.19	0.83	1.54±0.18	1.49±0.17	0.65	0.41
Ehrlich chromogen (nmol/g muscle)	6.03[ab]±.039	6.49[a]±.038	5.01[b]±.038	0.01	5.92±0.30	5.73±0.29	0.71	0.23
Pyridinoline (mol/mol collagen)	0.09±0.02	0.06±0.02	0.12±0.02	0.34	0.09±0.01	0.09±0.01	0.47	0.07
Pyridinoline (nmol/g muscle)	2.24±0.30	1.27±0.34	2.55±0.34	0.18	2.16±0.21	1.89±1.88	0.17	0.06

[1]Angus
[2] Charolais
[3] Kinsella
[ab] Means within the same row within the same main effect with different superscript letters are significantly (p<0.05)

Table 2-2 Effect of breed, residual feed intake and ageing on collagen characteristics of the m. *Gluteus medius* including significant interaction

	Breed				Residual feed intake			Ageing			Interactions			
Variables	AN[1]	CH[2]	KC[3]	p-value	High	Low	p-value	3dpm[4]	13dpm	p-value	BxR[5]	BxA[6]	RxA[7]	BxRxA[8]
n=	46	48	48	-	70	72	-	71	71	-	-	-	-	-
Cook loss (mg/g)	275.52±12.36	262.79±14.00	290.87±14.04	0.49	278.18±8.94	274.61±8.94	0.72	280.64±8.88	272.16±8.95	0.33	0.81	0.65	0.32	0.53
Cook time (sec/g)	4.11±0.33	4.16±0.33	4.09±0.32	0.98	4.07±0.24	4.17±0.23	0.91	4.38±0.25	3.86±0.22	0.07	0.59	0.30	0.15	0.43
Shear Force (kg)	3.74±0.38	3.46±0.46	3.74±0.46	0.88	3.59±0.26	3.70±0.26	0.42	4.05±0.26	3.25±0.26	<0.01	0.10	0.04	0.35	0.97
Core standard deviation	0.98±0.13	0.83±0.15	0.85±0.15	0.71	0.86±0.09	0.91±0.09	0.49	1.06±0.09	0.70±0.09	<0.01	0.89	0.03	0.61	0.70
Total Collagen (mg/g muscle)	4.31±0.43	4.21±0.45	4.15±0.45	0.91	4.35±0.42	4.10±0.41	0.44	4.79[a]±0.41	3.66[b]±0.41	<0.01	0.78	0.40	0.44	0.28
Collagen Solubility (%)	52.18±1.94	47.62±1.95	54.94±1.95	0.02	49.08±1.65	53.82±1.60	0.02	43.84±1.62	59.02±1.62	<0.01	0.27	0.01	0.11	0.54

[1] Angus
[2] Charolais
[3] Kinsella
[4] Days post-mortem
[5] BreedxRFI
[6] BreedxAgeing
[7] RFIxAgeing
[8] BreedxRFIxAgeing
[ab] Means within the same row within the same main effect with different superscript letters are significantly different ($p<0.05$)

Table 2-3 Pearson correlation analysis between meat quality traits and collagen characteristics in m. *Gluteus medius*

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1pH	1	0.08	-0.20	-0.09	0.05	0.06	-0.08	-0.02	-0.44*	-0.22	0.03	0.23	-0.26	-0.07	-0.28	-0.13	0.03	-0.18	-0.04	0.13	-0.18	-0.09	-0.06	0.03
2Temp (˚C)		1	0.22	-0.04	-0.13	0.13	-0.36*	0.02	-0.02	-0.16	0.60*	0.69*	0.06	0.15	-0.19	-0.26	0.17	0.11	0.13	-0.07	0.08	0.05	0.43*	-0.15
3Purge (mg/g)			1	-0.04	0.18	0.07	0.08	0.31	0.01	-0.13	0.17	0.14	0.29	0.22	0.33	0.06	0.08	0.18	-0.08	0.02	-0.06	-0.31	0.10	-0.14
4Fat (%)				1	0.18	-0.85*	-0.24	0.09	0.15	0.22	0.27	0.26	0.10	0.03	0.06	0.08	0.09	0.31	0.13	0.24	0.06	0.13	-0.08	-0.23
5Protein (%)					1	-0.43*	0.10	0.06	-0.04	0.00	-0.16	0.04	0.27	-0.18	0.31	0.01	0.13	0.19	0.00	0.29	0.05	-0.15	0.00	-0.01
6Moisture (%)						1	0.21	-0.20	-0.04	-0.16	-0.07	-0.11	-0.14	0.04	-0.05	-0.11	-0.12	-0.3	-0.16	-0.26	-0.07	0.00	0.21	0.13
7Drip loss (mg/g)							1	-0.17	-0.03	-0.02	-0.39*	-0.36*	-0.09	0.01	0.00	0.17	-0.18	0.06	-0.05	0.08	-0.10	-0.14	-0.28	0.13
8SarcomereLength (µm)								1	-0.23	-0.2	-0.05	-0.02	0.27	0.13	0.25	0.08	0.13	0.00	-0.01	0.00	-0.07	-0.09	-0.03	-0.07
9Perimysium (%)									1	0.80*	0.44*	0.00	0.03	-0.10	0.11	-0.13	-0.11	0.14	-0.01	0.13	0.21	0.16	0.07	-0.08
10EC[1] (nmol/muscle)										1	0.43*	-0.01	-0.08	-0.06	-0.01	0.03	-0.27	-0.01	-0.20	0.01	0.21	0.15	-0.05	-0.03
11PYR[2] (nmol/muscle)											1	0.79*	-0.01	0.10	-0.16	-0.20	0.05	0.00	0.06	-0.14	0.15	0.12	0.44*	-0.20
12PYR (mol/mol collagen)												1	0.08	0.08	-0.14	-0.26	0.26	0.05	0.18	-0.11	0.09	0.07	0.50*	-0.21
13Cook loss(mg/g)3dpm[3]													1	0.17	0.72*	0.02	0.71*	0.39*	0.41*	0.19	-0.18	-0.17	0.17	-0.03
14Cook loss (mg/g)13dpm														1	0.01	0.66*	0.03	0.40*	-0.04	-0.13	-0.06	-0.10	0.07	0.04
15Cook time (sec/g)3dpm															1	0.02	0.45*	0.19	0.28	0.23	-0.20	-0.13	0.22	-0.11
16Cook time (sec/g)13d																1	-0.17	0.17	-0.21	-0.19	0.04	0.08	-0.24	-0.05
17Shear Force (kg)3dpm																	1	0.44*	0.76*	0.25	-0.21	-0.12	0.22	0.03
18Shear Force (kg)13dpm																		1	0.30	0.57*	0.15	-0.05	0.03	-0.14
19CoreStdDev3dpm																			1	0.17	-0.23	0.03	0.19	0.23
20CoreStdDev13dpm																				1	-0.03	-0.06	-0.10	-0.13
21Total collagen (mg/g)3dpm																					1	0.03	-0.09	0.04
22Total collagen (mg/g)13dpm																						1	0.12	-0.26
23Collagen solubility (%)3dpm																							1	-0.23
24Collagen solubility (%)13dpi																								1

*Correlation is significant at (P<0.01)
[1]EC= Ehrlich Chromogen
[2]PYR = Pyridinoline
[3]dpm= Days postmortem

2.12 Figures

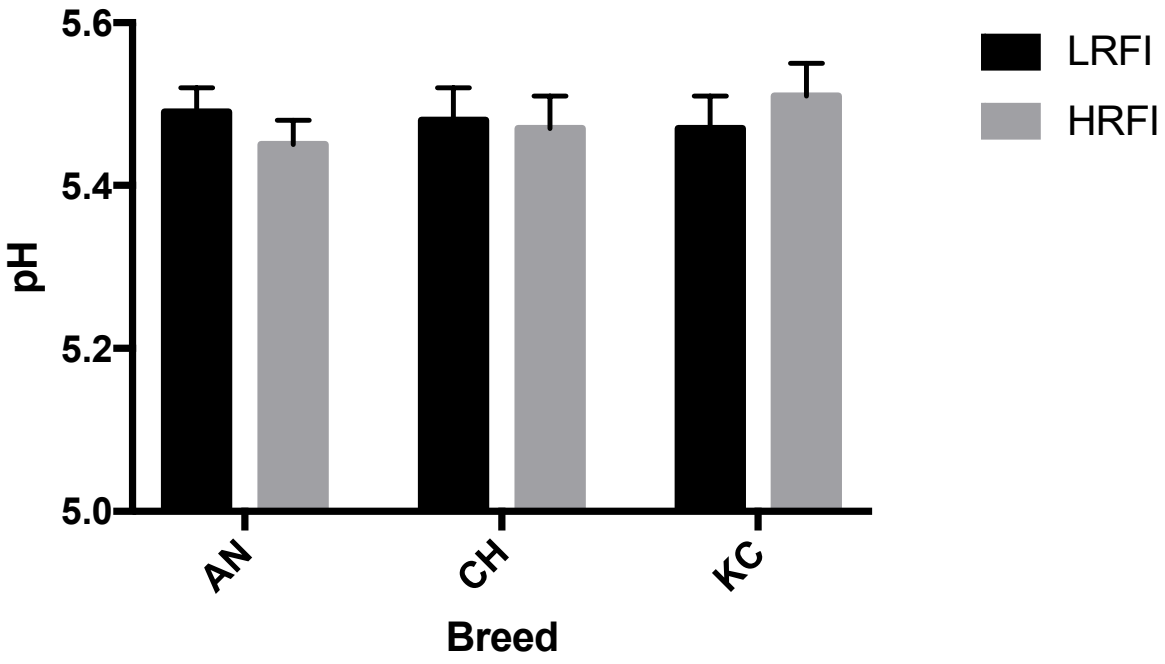

Figure 2.1 Interaction of breed and residual feed intake on the pH of the m. Gluteus medius.

Error bars are standard error of the mean (SEM). LRFI= low residual feed intake; HRFI= high residual feed intake. AN= Angus; CH= Charolais; KC= Kinsella Composite. [ab] Means different superscript letters are significantly different ($p<0.05$)

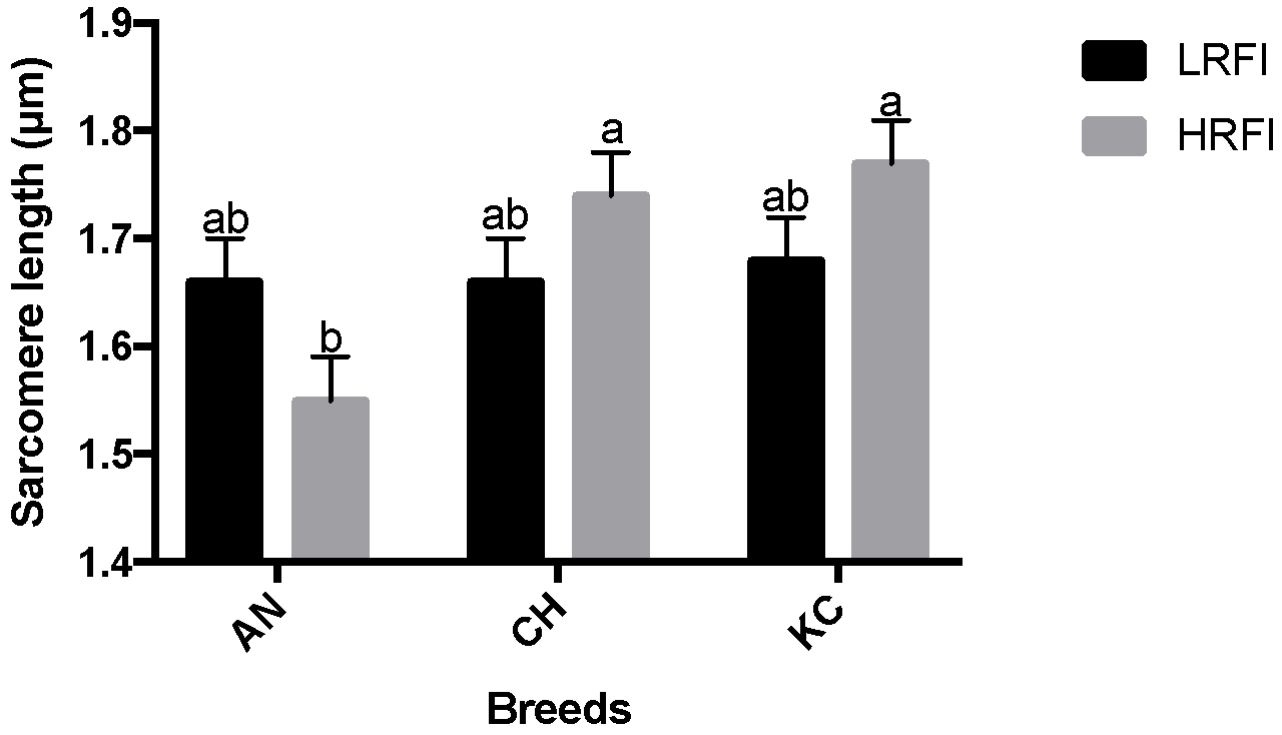

Figure 2.2 Interaction of breed and residual feed intake on sarcomere length of the m. *Gluteus medius*.

Error bars are standard error of the mean (SEM). LRFI= low residual feed intake; HRFI= high residual feed intake. AN= Angus; CH= Charolais; KC= Kinsella Composite. [ab] Means different superscript letters are significantly different ($p<0.05$).

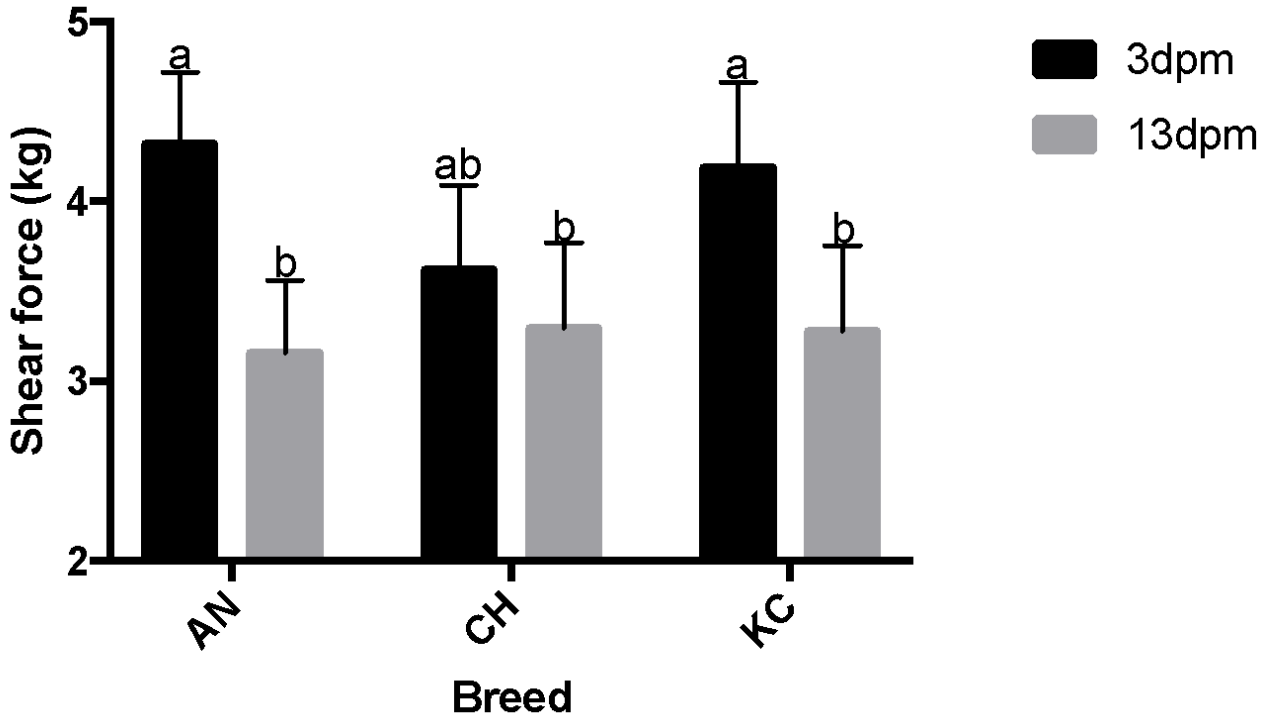

Figure 2.3 Interaction of breed and post-mortem ageing on Warner-Bratzler shear force of the m. *Gluteus medius.*

Error bars are standard error of the mean (SEM). 3dpm= days post-mortem; 13dpm= days post-mortem AN= Angus; CH= Charolais; KC= Kinsella Composite. [ab] Means different superscript letters are significantly different ($p<0.05$).

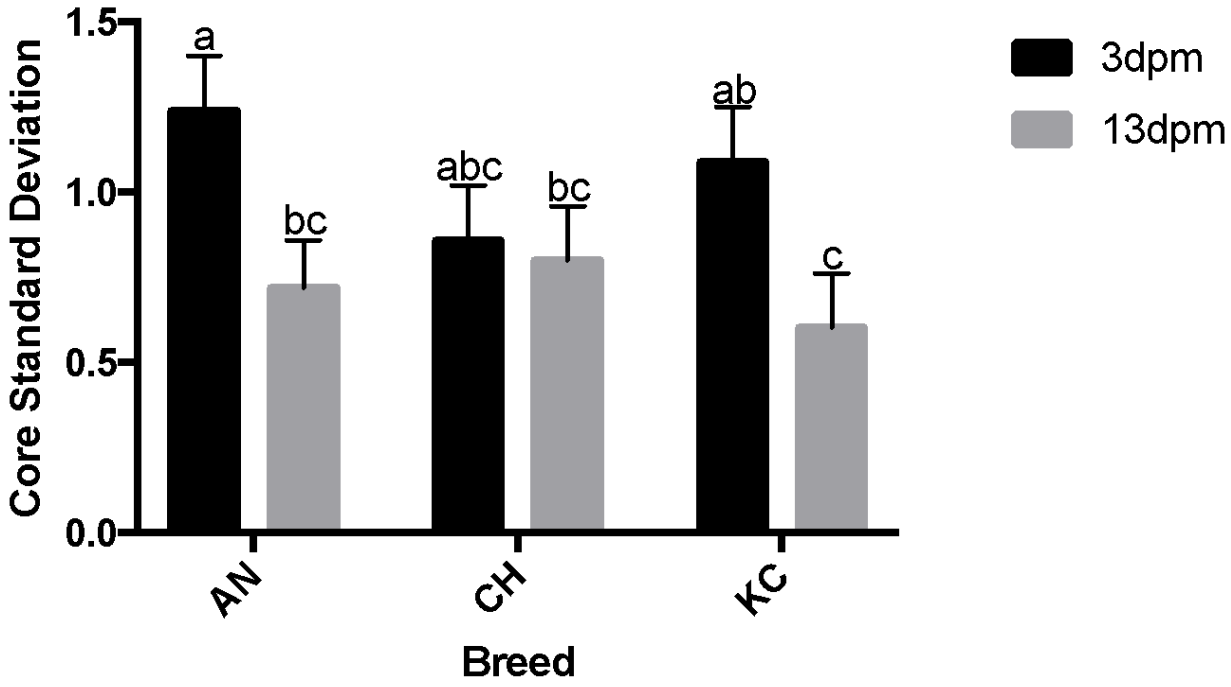

Figure 2.4 Interaction of breed and post-mortem ageing on the standard deviation of the shear force cores of the m*. Gluteus medius.*

Error bars are standard error of the mean (SEM). 3dpm= days post-mortem; 13dpm= days post-mortem AN= Angus; CH= Charolais; KC= Kinsella Composite. [ab] Means different superscript letters are significantly different ($p<0.05$).

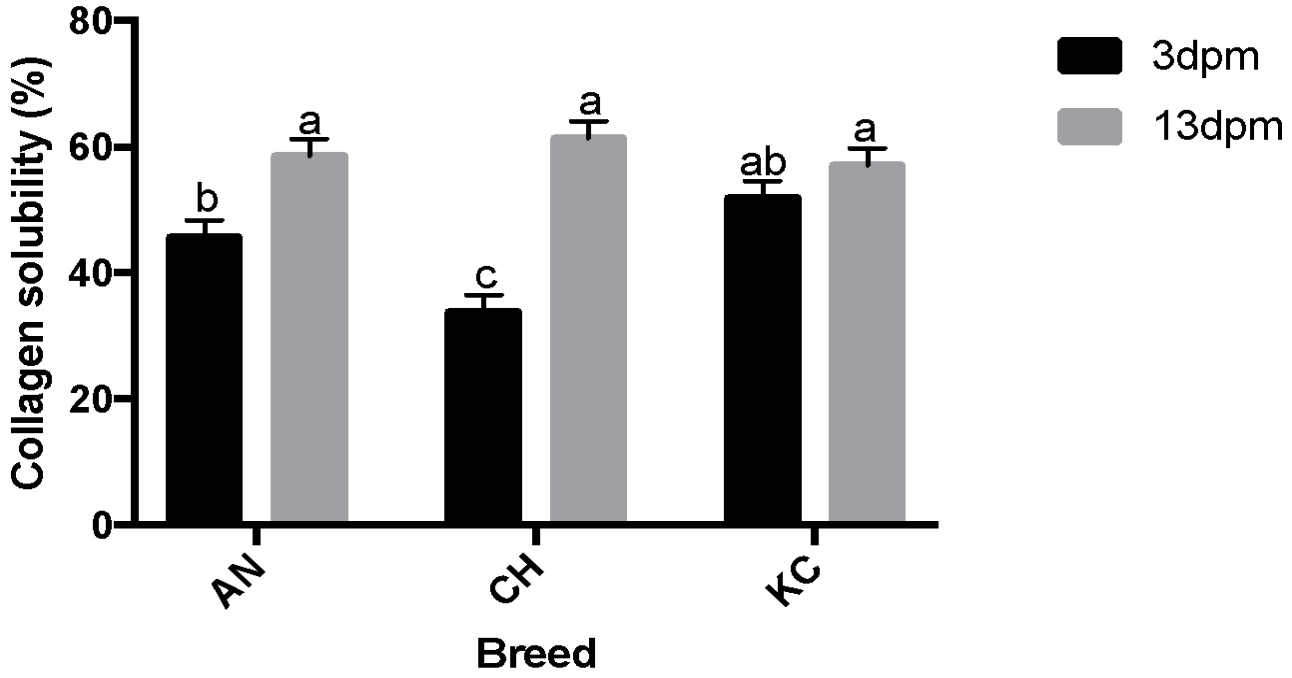

Figure 2.5 Interaction of breed and post-mortem ageing on Collagen solubility percentage of the m. *Gluteus medius.*

Error bars are standard error of the mean (SEM). 3dpm= days post-mortem; 13dpm= days post-mortem AN= Angus; CH= Charolais; KC= Kinsella Composite. [ab] Means different superscript letters are significantly different ($p<0.05$).

Chapter 3
Effects of cattle breed on the expression of genes involved in collagen synthesis and degradation and their relationship to meat quality and collagen characteristics of the m. *gluteus medius*

3.1 Introduction

Meat quality is measured mainly by the sensory attributes of colour, texture, juiciness and flavour, but the most important is tenderness (Bernard et al. 2007). These sensory factors describe meat quality, and can be used to detect differences in meat quality due to animal breed, production and slaughter practices, and muscle type (Hocquette et al. 2005). Meat tenderness is considered to be determined by the structure of myofibrillar and connective tissue proteins, and toughness due to the myofibrillar portion of muscle can usually be overcome by ageing carcasses. Ageing allows for the activity of endogenous proteolytic enzyme such as the calpains to occur, with toughness subsiding as a consequence of the breakdown of specific sarcomeric proteins (Koohmaraie and Geesink 2006).

McCormick (1994) ascribed some of meat toughness as coming from the stability and complexity of the cross-links of collagen, the main protein of connective tissue, with the amount of collagen also correlated with the toughness of meat because muscles with high amounts of collagen tend to have increased toughness (McCormick 1999; Nishimura 2010). Because myofibrillar proteins have been assumed to degrade more rapidly than collagen during the post mortem period, collagen has been attributed to forming the background toughness of meat (Purslow 2018). Reducing the background toughness of meat therefore would contribute to the reduction of the initial meat toughness, and reduce the ageing time postmortem required to produce beef that provides a high-quality eating experience. The formation of collagen and its crosslinks is tightly controlled by genetics and levels of gene expression. Understanding the function and characteristics of genes in these pathways, however, would allow for modification of the formation of collagen in muscle, the development and deposition of intramuscular fat, and the formation of collagen crosslinks so that their influence on meat quality could be controlled (Lehnert et al. 2006).

Expression of genes involved in collagen biosynthesis and degradation may vary between cattle breeds. Genetics govern the formation of muscle cells and determine muscle fiber type, which can then influence the amount of intramuscular collagen (Aalhus et al. 1991). Differences in gene expression between breeds may also affect intramuscular fat deposition, which may then affect the meat quality of a breed (Hocquette et al. 2010). With the advances in genomic technology and the advantages these methods confer to the understanding of biological mechanisms, the aim of this study was to analyze the level of expression of genes related to skeletal muscle collagen synthesis and degradation in the m. *gluteus medius* of three different breeds: purebred Charolais and Angus and cross-bred Angus (Kinsella Composite). This study then tested the null hypothesis that genes related to collagen biosynthesis and degradation differ between breeds, and that genes related to matrix degradation or new collagen synthesis will have increased expression in muscle that has increased collagen solubility.

3.2 Materials and methods

3.2.1 Animal source

Cattle for this study were sourced from the population described in Section 2.2.1 of this thesis. The beef steers were managed and slaughtered as described in Section 2.2 of this thesis and muscles harvested from their carcasses as described in Section 2.3 of this thesis. Meat quality measurements were conducted as described in Section 2.4 of this thesis, temperature, pH and purge as in Section 2.4.1, proximate analysis according to Section 2.4.2, sarcomere length as in Section 2.4.3, drip loss as in Section 2.4.4, cooking loss and cooking time according to Section 2.4.5, and Warner-Bratzler shear force (WBSF) as in Section 2.4.6. Collagen characteristics were determined as described in Section 2.5 of this thesis. Intramuscular connective tissue (IMCT) isolation was determined as described in Section 2.5.1, pyridinoline (PYR) quantification according to Section 2.5.2, total collagen quantification following Section 2.5.3 and collagen solubility as in Section 2.5.4. A total of 36 animals were selected, with 12 steers from each breed. Steers were selected within breed by selecting 6 high and 6 low RFI steers consisting of the 3 steers with the highest and 3 steers with the lowest increases in percentage of collagen heat solubility with post mortem ageing.

3.2.2 Muscle sampling

Samples for gene expression were removed from the m. *gluteus medius* of each carcass within 30 minutes of exsanguination. A 2 to 5 g sample of muscle was removed from the *gluteus medius* (top sirloin) over the ischium of the sacrum after the last sacral vertebrae on the right side of each carcass using a sterilized scalpel, with the core of muscle obtained from the dorsal aspect about 12 to 15 cm from the vertebrae. The samples of the muscle for RNA determination were placed in WhirlpakTM bags labelled with sample identification, frozen in liquid nitrogen and placed in dry ice until stored frozen at -80 ˚C until analysis.

3.2.3 Total RNA isolation

Muscle used for gene expression analysis was removed from -80 °C storage and immediately ground using a mortar and pestle with liquid nitrogen to maintain mRNA integrity. Once the tissue samples were ground into small pieces, an approximately 100 mg sample was transferred to a 2 mL ceramic tissue grinding beaded tube (Bertin Technologies, Montingyle Bretonneux, France) and stored for further homogenization. Total RNA was isolated using TRIzol® reagent (Invitrogen, Carlsbad, USA), using 1 mL TRIzol® to every 10 mg of tissue. Lysis and homogenization steps were then performed with the Cryolys® and Precellys® tissue homogenization system (Bertin Technologies, Montigny le Bretonneux, France).

Tissue was homogenized twice for 30 s at 6200 rpm (with a 10 second pause between two cycles) at 4˚C, and then the homogenization tubes were centrifuged at 12,000 g at 4 ˚C for 10 min. The supernatant was transferred to a new tube and RNA isolation was performed. Once the isolation was finished, the RNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, USA) and Qubit™(Invitrogen, Carlsbad, USA), and the integrity of the RNA was assessed with Agilent 2200 TapeStation Bioanalyzer (Agilent Technologies, Waldbronn, Germany). The RNA samples with integrity number (RIN) ≥ 7.0 were subjected to downstream qPCR analysis.

3.2.4 Reverse transcription and quantitative real-time PCR

After RNA isolation, the total RNA was prepared for reverse transcription by converting it to cDNA. Total RNA (2 µg) was reverse-transcribed into cDNA using iScript cDNA synthesis kit (Bioad, Hercules, CA) according to the manufacturer's instructions. A complementary DNA or cDNA was used as a template for the qPCR reaction amplified with real-time qPCR, which was performed using fast SYBR Green real-time-PCR Master Mix (Thermo Fisher Scientific, Waltham, USA). The qPCR was conducted in a 10 µL total

reaction volume containing 2 µL of cDNA, 5 µL of Fast SYBR Green Master Mix, and 1 µL of primers. The thermal cycling program was 95˚C for 20 s, followed by 40 cycles at 95˚C for 3 s, and then 60˚C for 30 s. Relative expression of each gene was calculated by the difference in CT values (delta CT) of the target and reference genes, with low delta CT values indicating high expression of the gene. The data was normalized using the 18S rRNA gene as the housekeeping gene. Twenty-four genes and primer sequences were selected base on a search of the literature (Du et al. 2013; Qi et al. 2016) and primer sequences were validated *in silico*. Primers for fourteen (14) genes were designed using the NCBI tool (Hamed, personal communication) using BLAST® on the website of the Information National Center for Biotechnology (2019) and primers and the amplicon sizes used in the real-time PCR are shown in Table 3-1.

3.2.5 Statistical analyses

Collagen characteristics and meat quality data were analyzed using a linear mixed effect model fitted in RStudio (RStudio Server Team 2016) as a split plot design where breed (Angus n=12, Charolais n=12 and Kinsella Angus crossbred n=12), RFI (low n=6, high n=6) and their interaction were tested in the main plot. Post mortem ageing time (days 3 and 13) was considered in the split plot. The experimental units were the GM muscle from each carcass for the whole plot and the steak that was aged in the split plot.

Relative gene expression levels in m. gluteus medius were analyzed using a two-way analysis of variance (ANOVA) (RStudio Server Team 2016) with breed (Angus n=12, Charolais n=12 and Kinsella n=12), level of collagen solubility (high n=6, low=6) and their interaction as the sources of variation. Statistical differences were deemed significant at $P<0.05$. Pearson's correlations between gene expression levels, collagen characteristics and meat quality measurements were performed using the package Hmisc in R studio (Version 4.0.2) with correlations considered significant at $P<0.01$.

3.3 Results

3.3.1 Meat quality

Purge loss was highest in Angus crossbred and lowest in the GM from the Angus (Table 3-2). There were no effects of breed, RFI or their interaction on GM temperature at fabrication, protein content, or drip loss (Table 3-2). There was a significant difference in sarcomere length due to breed, with the longest sarcomeres found in the GM from Kinsella composite steer carcasses and the shortest sarcomeres found in GM muscle from the Angus steer carcasses (Table 3-2). No significant effects of breed were observed

for intramuscular pH but there was an interaction (P<0.05) between breed and RFI on pH but the interaction was driven by the change in direction of the interaction slopes as there were no differences between the means (Figure 3.1). A significant interaction was observed between RFI and breed on intramuscular fat percentage, with selection for low RFI decreasing the fat content of GM in Angus steers only while the other breeds remain unchanged (Figure 3.2).

3.3.2 Collagen characteristics

There were no effects of breed, RFI or their interaction on the proportion of perimysium in the GM or Ehrlich's Chromogen concentration (Table 3-2). Breed and RFI interacted (P<0.01) on intramuscular PYR crosslink concentration, with the interaction showing no difference due to RFI within the Charolais and Kinsella crossbred steers, but within the Angus the concentration of PYR was increased in the high RFI steer muscle (Figure 3.3). For PYR crosslink density, the interaction between breed and RFI was significant, but showed no differences across the breed means, with the interaction being driven by the change in direction of the interaction slopes (Figure 3.4). No differences were found due to breed and RFI for any of the characteristics measured on both day 3 and 13, which included total collagen, collagen solubility, cooking loss, cooking time, shear force and the standard deviation of the shear force cores (Table 3-3). Mean shear force value and core standard deviation were also lower at day 13 post-mortem than at day 3 (Table 3-3).

Differences were found in total collagen due to post-mortem ageing (p<0.05), where the amount of collagen in the muscle was higher at day 3 post-mortem than at day 13 post-mortem. There was also a significant interaction (P=0.01) between breed and post mortem ageing on collagen solubility percentage in the GM, with collagen solubility in GM from Angus and Charolais carcasses increasing with ageing post-mortem but there was no effect of ageing on GM from Kinsella crossbred carcasses (Figure 3.5).

3.3.3 Gene expression analysis

A total of 38 genes were evaluated and normalized with a housekeeping gene. The statistical analysis of the gene expression levels of different genes indicated no significant differences due to level of collagen solubility or their interaction. There were 7 genes that exhibited differences due to breed (P<0.05) and these included lysyl oxidase (*LOX*), fibroblast growth factor receptor 1 (*FGFR1*), fibroblast growth factor 2 (*FGF2*), mother against decapentaplegic homolog 6 (*SMAD6*), tissue inhibitor of matrix metalloproteinases 3

(*TIMP3*), integrin subunit alpha 1 (*ITGA1*), AKT serine/threonine kinase 1 (*AKT1*) (Table 3-4). The highest expression for *LOX* was found in the Charolais (ΔCt = 9.03) and the Kinsella Angus crossbred (ΔCt=9.72), while the lowest expression was found in the Angus. Expression of *FGFR1* was the highest in the Kinsella Angus crossbred (ΔCt = 13.47) and lowest in the other two breeds. Expressions of *FGF2* (ΔCt = 9.60) and *SMAD6* (ΔCt = 13.01) were highest in the Kinsella Angus crossbred and the Charolais (ΔCt = 10.70 and ΔCt = 13.63, respectively) and lowest in the Angus. Expressions of *TIMP3* (ΔCt = 6.91) and *ITGA1*(ΔCt = 9.00) were highest in Angus and Kinsella Angus crossbred and lowest expression in the Charolais. AKT1 showed the highest expression in Angus (ΔCt = 10.11) (Table 3-4).

3.3.4 Pearson correlations

Pearson correlations between meat quality collagen characteristics and selected genes (Table 3-5) were considered significant at P<0.01. Measurements indicated that moisture and fat were negatively correlated (r=-0.91). PYR concentration was positively correlated with PYR density (r=0.75). PYR concentration in muscle was correlated with *ITGA1* (r=-0.52, P<0.01) and PYR density was correlated with *ITGA1* (r=-0.61, P<0.01). Pearson correlations were performed between genes to investigate any gene co-expression (Table 3-5). The analysis showed strong positive correlations between the expression of *LOX* and that of *FGFR1* (r=0.75), *FGF2* (r=0.94) *SMAD6* (r=0.86) P<0.01), and other positive strong correlations were found between expression of *FGFR1* and that of *FGF2* (r=0.78), *SMAD6* (r=0.78) and *ITGA1* (r=0.60, P<0.01). A strong correlation was found between expression of *FGF2* and *SMAD6* (r=0.91) and a moderate correction identified with the expression of *ITGA1* (r=0.51). A moderate positive correlation was identified between the expression of *SMAD6* and *ITGA1* (r=0.53) and moderate to strong positive correlations were found between expression of *TIMP3* and *ITGA1* (r=0.90), *AKT1* (r=0.47), *ITGA1* and AKT1 (r= 0.50).

3.4 Discussion

Selection of steers with differing phenotypes provides the opportunity for examination of gene expression in a population of known extremes (Lindholm-Perry et al. 2013; Berton et al. 2016). This approach assists with identifying differentially expressed genes quickly and in a small population (Ramayo-Caldas et al. 2012). Steers with extremes in collagen solubility were selected for gene expression analysis as results

from Chapter 2 indicated that post-mortem ageing was the treatment that affected collagen solubility most. In fact, post mortem ageing had the greatest impact on both meat quality and collagen characteristics in this chapter as it did in Chapter 2 with mean shear force values of cooked m. *gluteus medius* decreasing from day 3 to day 13 post mortem. The shear force results agree with those of Gruber et al. (2006) and Colle et al. (2016) and may be related to changes in Z-disk proteins enzymatically degraded by calpains (Koohmaraie 1994; Purslow 2014). Total collagen concentration decreased with post-mortem ageing as well, but was most likely due to a positional difference in the steaks used at each post-mortem ageing time, as was previously discussed in Chapter 2. Collagen solubility increased with post-mortem ageing and similar results were observed by others (Herring et al. 1967; Mills et al. 1989). Notably, the interaction between breed and post-mortem ageing for collagen solubility indicated that there was an increase in the collagen solubility of *gluteus medius* from all breeds, with that increase being greatest for the Charolais breed. Boccard et al. (1979) also mentioned changes in collagen solubility can be related to breed and type of muscle. Interestingly, regardless of the collagen solubility at day 3 post mortem, the mean collagen solubility for each breed was not different from each other by day 13 post mortem, suggesting that there may be a limit to how much collagen solubility can be gained with post mortem ageing. This result suggests that the cleavage points on collagen for matrix metalloproteinases may be finite.

This thesis is the first to relate the expression of genes involved in collagen synthesis and degradation with each other and with beef meat quality and collagen characteristics. In this study, the mRNA expression of *LOX* was greater in the GM of the Charolais and the Kinsella Angus crossbred than in the GM of the Angus and this may suggest that collagen synthesis and crosslinking were increased in these breeds at the time of slaughter (Gonzalez et al. 2014). The *LOX* protein catalyzes the oxidation reaction of peptidyl lysine with other amino acid groups to produce intermolecular collagen crosslinks (Kagan 1986). Polymorphisms of *LOX* and for the genes coding for calpastatin may change the beef tenderness (Drinkwater et al. 2006), as cattle that have low expression of *LOX* may have tender meat due to the late formation of collagen crosslinks (Park et al. 2018). The *LOX* gene expression in this study was strongly correlated with genes involved in the development and age-related changes of tissue, specifically *FGF2* (r=0.99), *FGFR1* (r=0.75), and *SMAD6* (r=0.87). The expression of the *LOX* gene most likely reflects collagen synthesis activity only, as lysyl oxidase initiates the formation of collagen crosslinks and so may not be indicating change to the

various collagen crosslinks concentrations (McCormick 1994). Lysyl oxidase does not direct the formation of specific types of collagen crosslinks as lysyl hydroxylase does, the upregulation of which has been linked to increased PYR concentration (Eyre et al. 1984). Increased expression of *LOX* may indicate increased synthesis of collagen (Sampson et al. 1984). Increased hydroxylation of proline however, has been linked to increased stability of the triple helix (Acevedo-Jake et al. 2017), which may affect the heat stability of the collagen independent of the collagen crosslink type.

The group of genes described as fibroblast growth factors (*FGF*) plays an important role in fibrogenesis. The results of this study on m. *gluteus medius* showed a higher expression of the genes *FGF2* and *FGFR1* in Angus crossbred animals than in the other two breeds. Results from this study differ from those by Wei et al. (2015) which showed that Angus cells had a greater response to *FGF2,* indicating higher cell proliferation fibrogenesis, with the animals in that study being 12-months old. This may mean that different breeds may have different levels of expression of these genes depending on their phenotype. Martínez et al. (2015) compared the *semitendinosus* and *longissimus thoracis et lumborum* muscles in beef cattle and found differences in expression of *FGF2*, which also suggests that muscles with distinct metabolic fiber types will differ in gene expression. Moreover, the differential expression of *FGF2* between bulls and steers did not mean significant differences in the levels of expression of other genes within the *FGF* family (Park et al. 2018). Other studies suggested that depending on the type of gene that is being expressed, the genes may be fibrogenic or lipogenic, and the texture of the meat will change with the development of either connective or adipose tissue (Du et al. 2013). In this thesis, given that the cattle were grown to a similar back fat and level of marbling, the genes may be myogenic rather than fibrogenic or adipogenic and may reflect increased proliferation of satellite cells (Pawlikowski et al. 2017). That *SMAD6* is upregulated supports this hypothesis as it is one of two inhibitors in the SMAD family (*SMAD6* and 7). *SMAD1* and 3 are genes induced by TGF-ß (Moustakas et al. 2001) and their phosphorylation by TGF-ß binding to the TGF-ß receptor II allows them to complex with to *SMAD4*, cross into the nucleus and promote the expression of fibrogenic genes (Suwanabol et al. 2011). The expression of mRNA of *SMAD6* in the m. *gluteus medius* was increased in the Charolais and Kinsella crossbred whereas the Angus had the lowest expression. This may indicate that breeds with increased expression of *SMAD6* may have decreased

formation of SMAD which may down-regulate fibrogenesis (Imamura et al. 1997) in favour of proliferation of satellite cell that can donate their nuclei to growing myofibres (Pawlikowski et al. 2017).

Matrix metalloproteinases (MMPs) are the enzymes that degrade components of connective tissue and these are active at neutral pH in muscle. These enzymes can be inhibited by tissue inhibitors of metalloproteinases (TIMPS), which bind to active sites of the MMPs (Malemud 2006). The expression of *TIMP3* in this study was increased in the GM of Angus and lowest in Charolais, and may indicate that collagen turnover was reduced in the GM of the Angus while that of Charolais was undergoing active re-modelling. Collagen turnover is usually increased during growth (Kehlet et al. 2018), and the reduced expression of *TIMP3* in the GM of the Charolais may indicate that the Charolais GM was undergoing active growth requiring the retention of existing and newly synthesized collagen. Charolais are considered a late maturing breed relative to Angus (Mao et al. 2013), but the turnover of muscle in the Charolais was not investigated in this thesis, precluding a firm conclusion.

AKT1 activates *ITGA1* as a result of adhesion to endothelial cells and is a mechanism by which fibroblasts regulate the assembly of extracellular matrix (Somanath et al. 2007). The gene expression in this analysis showed an increased expression in the GM of the Angus and low expression in the GM of Charolais for both genes. Park et al. (2018) conducted gene expression analysis on adipose tissue and found that an extracellular matrix receptor is involved in adipogenesis in cattle and that *ITGA1* was up- regulated in intramuscular fat. This may imply that Angus and Kinsella crossbred steers were actively depositing fat as they had the highest levels of expression.

The effect of breed and RFI were not expected to be different from that observed in the complete study detailed in Chapter 2. As was observed in the larger study in Chapter 2, RFI had no effect on most meat quality measurements with the exception of intramuscular pH where the means, although statistically difference, showed no meaningful biological difference as they were separated by only 0.01 pH units. Breed also had limited influence on meat quality in this subset of steers of the 71 steers described in Chapter 2. Although the sarcomere length in this study was affected by breed with longer sarcomeres found in the GM of Charolais and crossbred breed steer carcasses, there was no statistical difference between breeds for shear force, indicating that sarcomere length did not affect shear force. The studies of Whipple et al. (1990) and Stolowski et al. (2006) showed that mean sarcomere lengths were not affected by breed, although

these studies used both steers and heifers while only steers were used in this thesis, which could have influenced their results. Another trait that was significantly affected by breed was the purge loss, as increased purge loss could infer that there was a change in muscle cell integrity induced by low ultimate pH (Huff-Lonergan and Lonergan 2005), or by post-mortem proteolysis (Lonergan et al. 2010). The differences due to breed were not due to ultimate pH as there was no effect of breed on that measurement, and bag purge differences were not related to drip loss, suggesting that purge reflected water lost from cells during post mortem proteolysis that did not impact the ultimate water-holding capacity of the meat.

The interaction that was found between breed and RFI for the trivalent collagen crosslink pyridinoline showed an increase in the mean concentration of the crosslink in the GM from Angus steers that were not selected for low RFI. Dubost et al. (2013) also found differences in PYR concentration between the *longissimus thoracis, semimembranosus,* and *biceps femoris* in Aberdeen Angus but no differences due to muscle type in Limousin and Blond d'Aquitaine. These authors also observed the same pattern of significance due to breed for total collagen and collagen solubility, supporting the finding of this thesis that PYR concentration can vary by breed. Interestingly, there was no correlation between total collagen content and shear force, or PYR and EC and shear force indicating that total collagen did not influence cooked GM toughness as estimated using WBSF. These results agree with those of Girard et al. (2011, 2012), who found that GM toughness was determined more by myofibrillar protein than collagen. Despite these results, collagen characterization of the GM was warranted as Girard et al. (2011, 2012) also found that collagen heat solubility of the GM declined with steer age and was accompanied by an increase in Warner-Bratzler shear force.

3.5 Conclusion

The hypothesis that the expression of genes involved with collagen biosynthesis and degradation is related to collagen heat solubility was not accepted, but this study revealed co-expression patterns in the gene expressions that require further investigation. Of the 38 genes investigated during the relative gene expression level analysis, 7 genes were statistically different across breeds, suggesting that manipulation of collagen synthesis and degradation may be possible through selection of breed.

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3.7 Tables

Table 3-1 Primer sequences used in real time qPCR

Gene name (symbol)	Sequence (5' - 3')	Amplicon size, bp	Function
Metallopeptidase Inhibitor 1(*TIMP1*)	Forward GATGTCGTCATCAGGGCCAA Reverse GGGTGTAGATGAACCGGATG	145	CD[1]
Metallopeptidase Inhibitor 3(*TIMP3*)	Forward GACATCGTGATCCGAGCCAA Reverse TGGGGCATCTTGGTGAATCC	119	CD
Matrix Metallopeptidase 13 (MMP13)	Forward TTGTTGGTCTCTGCCCCTTC Reverse AATCACAGAGCTTGCTGCAG	148	CD
Matrix Metallopeptidase 2 (*MMP2)*	Forward TGATGGCGCCCATTTATACC Reverse GCCGGTGCCAGTATCAATGT	110	CD
Matrix Metallopeptidase 8 (*MMP8)*	Forward TTTCCTGTTGCTGCCCATGA Reverse ATGCAGTGAGTAGCTGCTGG	117	CD
Matrix Metallopeptidase 1 (*MMP1)*	Forward ACTTGTACCGGGTGGCAGCG Reverse GTTTGGGGGCCGACTGGCTG	190	CD
Integrin Subunit Alpha 1 (*ITGA1*)	Forward CACCAACCCAAAAGGAGGGT Reverse TGGGGCTGACATCAGAACAG	101	CS[2]
Integrin Subunit Alpha11 (*ITGA11*)	Forward GCCTACAGCACCGTCCTAAA Reverse TCGATGCTGCCATCTGAGTC	89	CS
Integrin Subunit Beta 1 (*ITGB1)*	Forward GCCTTGCATTGCTGCTGATT Reverse CAGTTGTCACAGCACTCTTG	138	CS
Collagen Type III Alpha 1 Chain (*COL3A1)*	Forward ATGTTGTGCAGTTTGCCCAC Reverse AGGACCAGGATCGCCATTTC	127	CS
Lysyl Oxidase (*LOX*)	Forward ACACACACAGGGCTTGAGTC Reverse TCAGGCACCAAATAGCTGGG	138	CS
Fibronectin 1 (*FN1)*	Forward TCAGAGACGGGCAAGAGAGA Reverse AGTAATGTCGGGAGTGGTGC	146	CS
Fibroblast Growth Factor Receptor 1 (*FGFR1)*	Forward GGCAGTGACACCACCTACTT Reverse AGCCACGGGGTTTGGTTTG	129	CS
Fibroblast Growth Factor 2 (*FGF2)*	Forward CCACTTCAAGGACCCCAAGC Reverse GTAGTTTGATGTGTGGGTCGC	116	CS
SMAD Family Member 6 (*SMAD6)*	Forward AGGATTCCCAGCAGCTCTTTG Reverse AATCCCTGGAATTGCATGCA	141	CS

Collagen Type VI Alpha 1 Chain (*COL6A1)*	Forward GCTCTTCTCGGATGGCAACT Reverse GCCGAAGACCACGTCATACT	90	CS
Insulin Like Growth Factor 1(*IGF1)*	Forward CCATCTCCCTGGATTTCTTTTTG Reverse GAAGAGATGCGAGGAGGATGTG	177	CS
Procollagen-Lysinem2-Oxoglutarate 5-Dioxygenase 1 (*PLOD1)*	Forward TCCACTACCCCCAAAAACGG Reverse GGATCCACGCTGAAGTAGT	218	CS
Prolyl 4-Hydroxylase Subunit Alpha1 (*P4HA1)*	Forward CAAAAGTGCCTGGCTGTCTG Reverse TGGCTCATCTTTCCGTGCAA	110	CS
Paired Like Homeodomain 2 (*PITX2)*	Forward CGAAGACCCGTCCAAGAAGA Reverse TAGCGGTTCCTCTGGAAAGTG	270	CS
SMAD Family Member 3 (*SMAD3)*	Forward TGAAGCGAAGTTTGGGCGG Reverse GCAGGATGGACGACATGGTT	136	CS
SMAD Family Member 4 (*SMAD4)*	Forward CCCCATCCCGGACATTACT Reverse CGATCTCCTCCAGAAGGGTCTA	200	CS
Collagen Type I Alpha 1 Chain (*COL1A1)*	Forward CGAGGAAATGATGGTGCGAC Reverse CTTCACCCTTAGCACCCACAG	100	CS
SMAD Family member 7 (*SMAD7)*	Forward CCCATCACCTTAGCCGACTC Reverse CTGGACAGTCTGCTGTGGATT	108	CS
Collagen Type V Alpha 1 (*COL5A1)*	Forward GACTTCCCTGACGGCGAATA Reverse TTCTTGTCGGGGAAGACGC	113	CS
Collagen Type III Alpha 1 (*COL3A1)*	Forward ATTGGCCCTGTTTGCTTTTTATAA Reverse TGGTCACTTGTACTGGCTGACAA	109	CS
Collagen Type III Alpha 1 (*COL3A1)*-2	Forward GCAAAACAGTCTTCCAGTATCAAACA Reverse GCCAATGTCCGCACCAA	109	CS
Collagen Type V Alpha 2 (*COL5A2)*	Forward AGCTGGGATGGACTTACTCTACTTG Reverse TAATAACAATACATTTTAGCCTGCCTAT	109	CS
Prolyl 4-Hydroxylase Subunit Alpha 1 (*P4HA1)*-2	Forward GGACTGTTTGAGTTGGGCAAA Reverse CGGTAGAAACCTCGCCTTCA	109	CS
AKT Serine/Threonine Kinase 1 (*AKT1)*-2	Forward ATCATGCAGCACCGATTCTTC Reverse AAATACCTGGTGTCCGTCTCAGAT	109	CS
AKT Serine/Threonine Kinase 1 (*AKT1)*-3	Forward ATCATGCAGCACCGATTCTTC Reverse AAATACCTGGTGTCCGTCTCAGA	109	CS
SMAD Family Member (*SMAD2)*	Forward GGAACTGCCCCCTCTGGAT Reverse ATCCAGGAGGTGGCGTTTCT	109	CS
Forkhead Box O1 (*FOXO1)*	Forward CATGTTTATCGAGCGCTTGGA Reverse GAAGCTTTGGTTGGGCAAAA	111	CS

Forkhead Box O1 (*FOXO1)*-2	Forward AAGACGCAGTGTGGCTTATGTAGA Reverse GGGTCTGTCTGCGTCTGGAT	118	CS
Forkhead Box O1 (*FOXO1)*-3	Forward AAAGACGCAGTGTGGCTTATGTAG Reverse GGGTCTGTCTGCGTCTGGAT	118	CS
Glycogen Synthase Kinase 3 Beta (*GSK3B)*	Forward TACCAAATGGGCGAGACACA Reverse TGCTTGAATCCGAGCATGAG	109	CS
RNA, *18S r*ibosomal 1 (*RNA18S1)*	Forward GTAACCGTTGAACCCCATT Reverse CCATCCAATCGGTAGTAGCG	-	-

[1]Collagen degradation
[2]Collagen synthesis

Table 3-2 Effects of breed, RFI and their interaction on meat quality and perimysium characteristics of the m. Gluteus medius.

	Breeds				Residual Feed Intake			Interaction
Variables	AN[1]	**CH[2]**	**KC[3]**	p-value	HRFI	LRFI	p-value	BreedxRFI
n=	12	12	12	-	18	18	-	-
pH	5.48±0.03	5.49±0.04	5.50±0.04	0.93	5.48±0.02	5.49±0.02	0.21	<0.01
Temp (˚C)	2.34±0.33	1.65±0.38	3.47±0.39	0.08	2.46±0.24	2.51±0.23	0.80	0.86
Purge (%)	6.29^{b}±0.73	7.11^{ab}±0.73	9.43^{a}±0.73	0.01	8.02±0.60	7.19±0.60	0.23	0.70
Fat (%)	4.29±0.46	3.86±0.53	3.60±0.53	0.64	4.11±0.32	3.72±0.31	0.15	<0.01
Protein (%)	20.76±0.21	21.27±0.21	20.72±0.21	0.30	20.81±0.17	21.02±0.17	0.39	0.56
Moisture (%)	73.17±0.34	72.92±0.38	73.52±0.38	0.58	73.15±0.25	73.25±0.24	0.71	0.06
Drip loss (mg/g)	42.14±2.79	47.11±2.92	42.16±2.92	0.46	44.51±2.19	43.10±2.15	0.62	0.94
Sarcomere length (µm)	1.58^{b}±0.03	1.71^{a}±0.03	1.73^{a}±0.03	0.02	1.69±0.03	1.67±0.03	0.73	0.42
Perimysium (%)	1.42±0.13	1.43±0.13	1.34±0.13	0.89	1.50±0.11	1.29±0.11	0.21	0.32
EC[4] (nmol/g muscle)	5.90±0.58	6.45±0.58	5.04±0.58	0.24	6.11±0.47	5.48±0.47	0.36	0.36
PYR[5] (mol/mol collagen)	0.10±0.02	0.05±0.02	0.12±0.02	0.30	0.10±0.01	0.09±0.01	0.511	<0.01
PYR (nmol/g muscle)	2.55±0.40	1.20±0.45	2.62±0.45	0.15	2.33±0.30	1.92±0.29	0.19	0.01

[1]AN= Angus
[2]CH= Charolais
[3]KC= Kinsella composite
[4]EC= Ehrlich Chromogen
[5]PYR = Pyridinoline
[ab] Means within the same row within the same main effect with different superscript letters are significantly different (P<0.05)

Table 3-3 Analysis of breed, RFI and post mortem ageing on collagen and meat quality characteristics of the m. *Gluteus medius*

	Breeds				Residual feed intake			Post-mortem Ageing		
Variables	AN[1]	CH[2]	KC[3]	p-value	HRFI	LRFI	p-value	Day 3	Day13	p-value
n=	12	12	12	-	18	18	-	18	18	-
Cook loss (mg/g muscle)	283.60 ±11.54	256.95±11.71	291.33±11.71	0.23	287.81±9.30	266.78±9.24	0.10	277.45±9.26	277.14±9.26	0.98
Cook time sec/g muscle	4.03±0.42	4.10±0.35	3.78±0.33	0.79	3.9±0.32	4.04±0.29	0.74	4.08±0.33	3.85±0.27	0.60
Shear force (kg)	3.90±0.40	3.54±0.48	3.53±0.48	0.80	3.62±0.28	3.69±0.27	0.70	4.04±0.28^{b}	3.27±0.28^{a}	<0.01
Core standard deviation	1.02±0.12	0.83±0.13	0.70±0.13	0.34	0.87±0.09	0.83±0.09	0.67	0.99±0.09^{b}	0.71±0.09^{a}	0.01
Total collagen (mg/g muscle)	4.34±0.58	4.20±0.62	4.11±0.62	0.96	4.30±0.44	4.14±0.43	0.74	4.75±0.43^{b}	3.69±0.43^{a}	0.03
Collagen solubility (%)	50.49±3.11	45.44±3.11	52.90±3.11	0.23	48.07±2.54	51.15±2.54	0.39	42.35^{b}±2.54	56.87^{a}±2.54	<0.01

[1]AN= Angus
[2]CH= Charolais
[3]KC= Kinsella composite
[ab] Means within the same row within the same main effect with different superscript letters are significantly different (P<0.05).

Table 3-4 Statistical analysis of breed and collagen solubility on gene expression of 38 genes using two-way ANOVA on m. *Gluteus medius.*

Variables	Breeds					Solubility			
	AN[1]	CH[2]	KC[3]	SEM[4]	P-value	High	Low	SEM	P-value
n=	12	12	12	-		18	18	-	-
COL5A1	13.32[6]	14.34	13.65	0.48	0.39	13.769	13.77	0.39	0.99
MMP-1	10.43	8.47	9.10	0.66	0.22	9.67	8.99	0.54	0.38
COL1A1	13.15	11.43	11.53	0.69	0.29	12.18	11.89	0.57	0.72
SMAD7	16.16	13.45	14.24	0.67	0.07	14.73	14.50	0.55	0.76
COL3A1	12.58	11.62	10.77	0.47	0.09	11.85	11.47	0.39	0.49
MMP-13	10.62	7.99	8.07	0.70	0.06	9.14	8.64	0.57	0.55
LOX	12.55[a]	9.03[b]	9.72[b]	0.70	0.02	10.51	10.35	0.57	0.84
FN1	10.44	9.38	8.86	0.41	0.09	9.75	9.37	0.34	0.44
FGFR1	15.08[a]	14.74[a]	13.47[b]	0.33	0.03	14.56	14.30	0.28	0.51
FGF2	13.56[a]	10.70[b]	9.60[b]	0.75	<0.01	11.67	10.91	0.63	0.39
SMAD6	15.84[a]	13.63[b]	13.01[b]	0.56	<0.01	14.46	13.86	0.47	0.36
COL6A1	14.56	13.53	13.59	0.53	0.39	13.96	13.83	0.43	0.84
MMP8	9.38	8.07	7.50	0.82	0.36	8.57	8.06	0.67	0.59
MMP2	12.86	13.57	12.54	0.39	0.29	13.07	12.91	0.31	0.72
IGF	13.23	14.06	11.47	0.82	0.19	13.06	12.77	0.67	0.75
PLOD1	11.30	9.43	8.89	0.83	0.22	10.06	9.68	0.68	0.69
P4HA1	10.56	10.00	8.50	0.59	0.12	9.65	9.73	0.48	0.91
PITX2	12.19	11.48	10.38	0.42	0.05	11.43	11.27	0.34	0.75
TIMP1	10.40	11.36	9.75	0.58	0.28	10.75	10.26	0.47	0.46
TIMP3	6.91[b]	9.24[a]	8.12[ab]	0.40	0.01	8.14	8.04	0.33	0.83
ITGA1	9.00[b]	11.18[a]	9.17[b]	0.44	0.01	9.93	9.64	0.36	0.57

ITGA11	11.34	7.28	10.79	2.57	0.52	9.82	9.78	0.72	0.98
ITGB1	7.18	8.23	6.96	0.53	0.30	7.67	7.24	0.43	0.49
COL3A1-1	3.91	2.65	3.73	0.64	0.40	3.97	2.88	0.52	0.15
COL3A1-2	5.45	3.90	4.32	0.66	0.34	5.03	4.09	0.54	0.23
COL5A2-1	8.73	7.88	6.87	0.73	0.30	8.36	7.29	0.60	0.21
COL5A2-2	9.69	8.94	8.58	0.49	0.36	9.36	8.78	0.40	0.31
COL5A2-3	10.54	8.25	10.09	0.55	0.05	10.01	9.25	0.45	0.24
AKT1-2	10.11[b]	11.72[a]	12.06[a]	0.40	0.01	11.32	11.27	0.33	0.91
AKT1-3	11.42	11.51	12.02	0.33	0.45	11.94	11.35	0.26	0.12
SMAD2-3	6.67	7.05	9.35	0.75	0.09	8.12	7.26	0.60	0.31
FOXO-1	8.52	8.71	8.48	0.45	0.92	8.77	8.38	0.36	0.44
FOXO1-2	7.60	7.40	7.18	0.50	0.88	13.20	13.07	0.44	0.24
FOXO1-3	7.83	7.59	7.25	0.51	0.77	7.92	7.20	0.43	0.24
SMAD3	16.62	16.61	14.57	0.78	0.22	16.56	15.30	0.64	0.17
SMAD4	11.72	11.54	11.49	0.26	0.84	11.57	11.60	0.21	0.92
GSK3B-1	10.69	9.92	10.53	0.44	0.48	10.63	10.13	0.36	0.33
GSK3B-2	11.10	11.50	10.44	0.41	0.30	11.39	10.64	0.33	0.12

Lower ΔCT values indicates high expression due to normalization against reference gene
[1]AN= Angus
[2]CH= Charolais
[3]KC= Kinsella composite
[4]Standar error of the mean
[5]Not significant
[6]ΔCT value
[ab] Means within the same row within the same main effect with different superscript letters are significantly different at p<0.01.
No significant interaction between breed and collagen solubility
The p-values were adjusted using FDR (false discovery rate) p<0.01

Table 3-5 Pearson correlation analysis between expression of selected gene, meat quality and collagen characteristic in m. *Gluteus medius*

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1 pH	1.00	0.00	-0.36	-0.20	0.05	0.25	-0.13	-0.28	-0.49*	-0.39	-0.15	0.14	-0.11	-0.29	-0.08	-0.31	0.22	-0.34	0.18	0.14	-0.22	0.01	0.00	0.16	0.40	0.33	0.38	0.26	-0.22	-0.02	0.18
2 Temp (˚C)		1.00	0.25	0.00	-0.40	0.12	-0.56*	0.03	0.09	-0.06	0.61*	0.61*	-0.12	0.23	-0.29	-0.31	-0.14	-0.05	-0.09	-0.22	0.14	0.09	0.46	-0.27	-0.07	-0.35	-0.23	-0.21	-0.27	-0.48	-0.05
3 Purge (mg/g)			1.00	-0.10	-0.03	0.03	0.34	0.52*	0.14	0.00	0.15	0.08	0.22	0.35	0.32	0.12	-0.10	0.21	-0.10	0.04	-0.05	-0.27	0.08	-0.24	-0.27	-0.36	-0.24	-0.25	0.01	-0.14	0.07
4 Fat (%)				1.00	0.22	-0.91*	-0.13	-0.07	0.25	0.29	0.33	0.37	0.10	0.08	0.01	0.10	0.20	0.41	0.38	0.19	0.02	0.20	-0.03	-0.26	0.05	0.10	0.07	0.16	-0.03	-0.05	-0.21
5 Protein (%)					1.00	-0.44	0.18	-0.06	-0.04	-0.04	-0.24	-0.02	0.21	-0.34	0.28	0.09	0.15	0.14	0.07	0.24	0.07	0.09	-0.06	-0.20	-0.07	0.26	0.05	0.06	0.41	0.38	0.10
6 Moisture (%)						1.00	0.05	-0.03	-0.16	-0.21	-0.12	-0.17	-0.12	0.00	-0.01	-0.20	-0.19	-0.42	-0.33	-0.22	-0.04	-0.13	0.22	0.23	0.07	-0.08	-0.01	-0.08	-0.19	-0.17	0.13
7 Drip loss (mg/g)							1.00	-0.06	-0.03	-0.03	-0.44	-0.48	-0.03	-0.08	0.15	0.13	-0.10	0.11	0.05	0.17	-0.11	-0.25	-0.36	0.28	-0.27	-0.05	-0.16	-0.18	0.07	0.08	0.05
8 SarcomereLength (µm)								1.00	-0.03	-0.01	-0.10	-0.12	0.37	0.17	0.37	0.04	0.18	0.09	-0.05	0.08	-0.12	-0.45	-0.08	-0.12	-0.07	-0.15	-0.04	-0.02	0.28	0.17	0.08
9 Perimysium (%)									1.00	0.88*	0.59*	0.10	0.07	-0.05	0.15	-0.04	-0.26	0.13	-0.17	0.07	0.30	0.24	0.12	-0.39	-0.08	0.04	-0.09	0.05	0.00	-0.12	-0.46
10 EC[1] (nmol/muscle)										1.00	0.57*	0.12	0.00	-0.01	0.06	0.09	-0.33	0.00	-0.30	-0.11	0.27	0.31	0.03	-0.24	0.00	0.18	-0.03	0.06	0.06	-0.02	-0.48
11 PYR[2] (nmol/muscle)											1.00	0.78*	0.00	0.27	-0.12	-0.07	-0.19	-0.06	-0.15	-0.24	0.21	0.21	0.50*	-0.40	0.11	-0.08	-0.04	0.00	-0.36	-0.51*	-0.45
12 PYR (mol/mol collagen)												1.00	0.02	0.28	-0.15	-0.16	0.06	0.00	0.03	-0.21	0.14	0.18	0.64*	-0.34	0.07	-0.19	-0.09	-0.10	-0.45	-0.61*	-0.26
13 Cook loss(mg/g)3dpm[3]													1.00	0.25	0.69*	0.15	0.73*	0.36	0.23	0.25	-0.26	-0.31	0.17	-0.19	0.13	-0.02	0.21	0.17	0.01	0.06	0.14
14 Cook loss (mg/g)13dpm														1.00	-0.12	0.55*	0.08	0.39	0.05	-0.11	0.01	-0.13	0.06	0.24	-0.17	-0.35	-0.22	-0.20	-0.36	-0.31	-0.14
15 Cook time (sec/g)3dpm															1.00	0.00	0.41	0.20	0.15	0.42	-0.28	-0.09	0.25	-0.37	0.13	0.07	0.22	0.20	0.04	0.10	0.01
16 Cook time (sec/g)13d																1.00	0.00	0.21	-0.08	-0.17	0.17	-0.01	-0.28	0.30	-0.05	0.15	-0.01	-0.07	0.07	0.23	0.02
17 Shear Force (kg)3dpm																	1.00	0.42	0.57*	0.39	-0.25	-0.26	0.13	-0.12	0.22	0.02	0.32	0.29	-0.14	-0.01	0.16
18 Shear Force (kg)13dpm																		1.00	0.33	0.61*	0.23	0.15	-0.05	-0.21	-0.47	-0.35	-0.32	-0.16	-0.23	-0.18	-0.17
19 CoreStdDev3dpm																			1.00	0.37	-0.23	-0.19	-0.08	0.11	0.14	-0.03	0.20	0.26	-0.16	-0.05	0.19
20 CoreStdDev13dpm																				1.00	-0.03	0.00	-0.14	-0.27	-0.27	-0.06	-0.10	0.00	-0.27	-0.10	-0.28
21 Total collagen (mg/g)3dpm																					1.00	0.21	-0.11	0.07	-0.32	-0.15	-0.33	-0.31	-0.15	-0.28	-0.30
22 Total collagen (mg/g)13dpm																						1.00	0.30	-0.37	-0.04	0.08	-0.09	-0.02	-0.03	0.00	-0.23
23 Collagen solubility (%)3dpm																							1.00	-0.59*	0.12	-0.13	-0.02	-0.02	-0.31	-0.45	-0.13
24 Collagen solubility (%)13dpm																								1.00	0.06	0.06	0.00	-0.05	0.00	0.08	0.20
25 *LOX*																									1.00	0.75*	0.94*	0.85*	0.21	0.36	0.33
26 *FGFR1*																										1.00	0.78*	0.78*	0.42	.060*	0.23
27 *FGF2*																											1.00	0.93*	0.32	0.51*	0.36
28 *SMAD6*																												1.00	0.35	0.53*	0.28
29 *TIMP3*																													1.00	0.89*	0.49*
30 *ITGA1*																														1.00	0.50*
31 *AKT12*																															1.00

*Correlation is significant at (P<0.01)
[1]Ehrlich Chromogen
[2]Pyridinoline
[3]Days post mortem

3.8 Figures

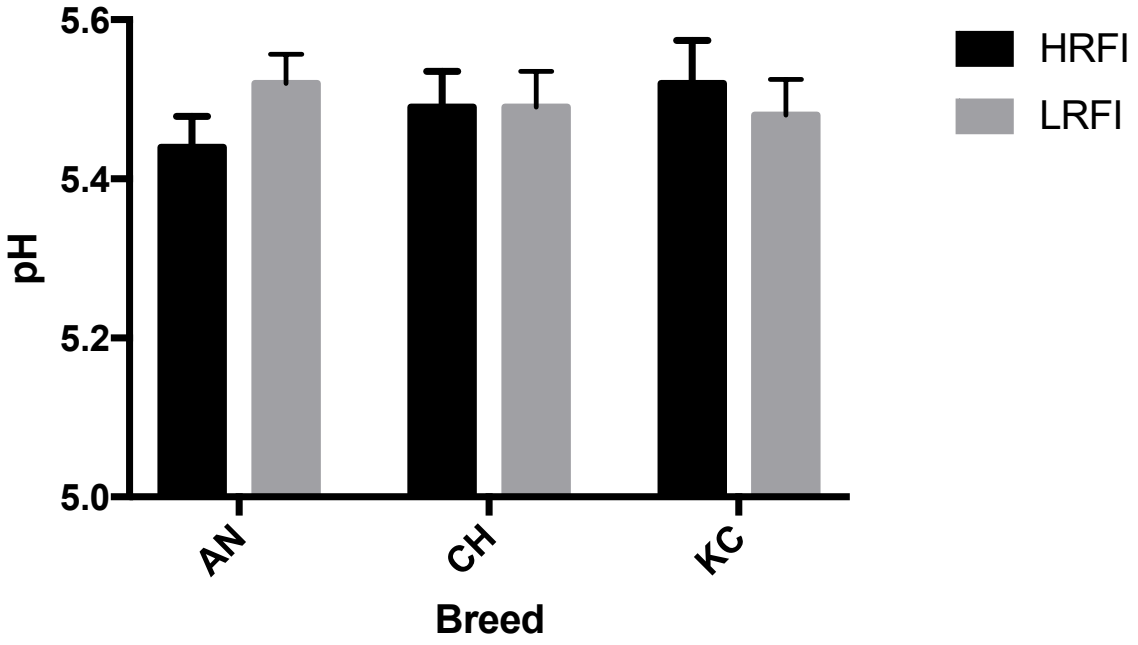

Figure 3.1 Interaction of breed and residual feed intake on the intramuscular pH of the *m. gluteus medius*

Error bars are standard error of the mean (SEM). LRFI= low residual feed intake; HRFI= high residual feed intake; AN= Angus; CH= Charolais; KC= Kinsella Composite. [ab] Means different superscript letters are significantly different ($p<0.05$).

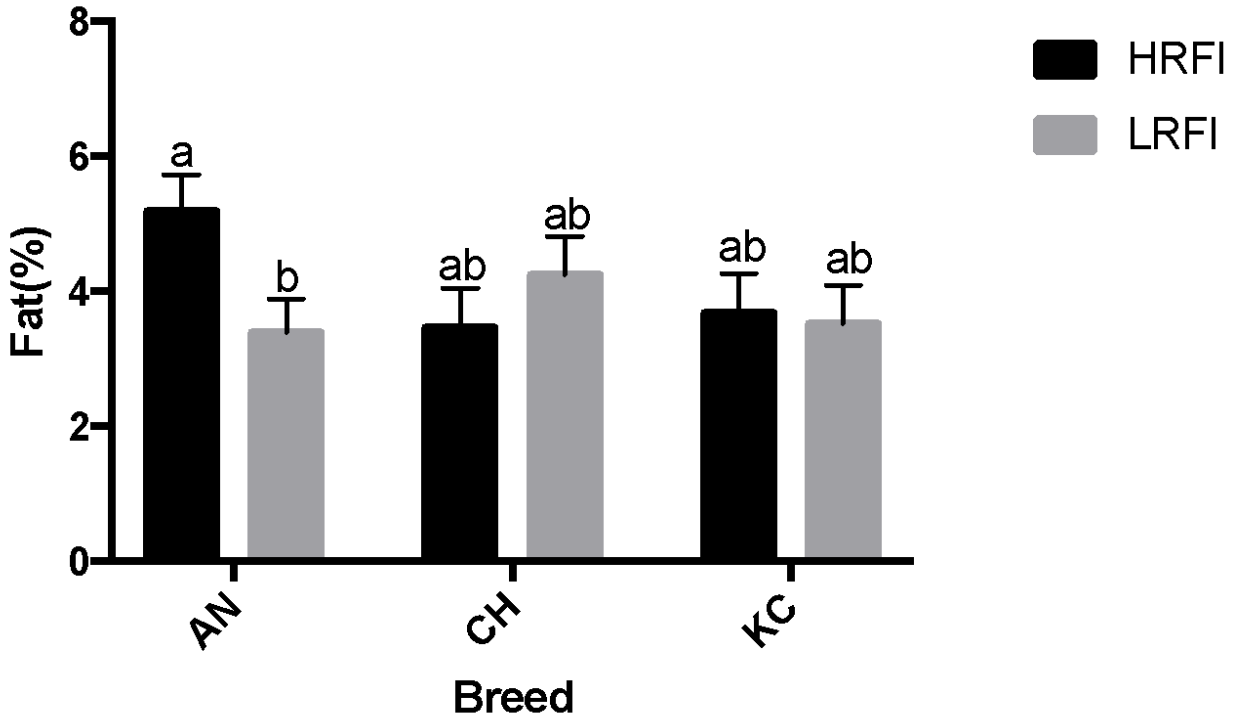

Figure 3.2 Interaction of breed and residual feed intake on intramuscular crude fat percentage of the m. *gluteus medius*.

Error bars are standard error of the mean (SEM). LRFI= low residual feed intake; HRFI= high residual feed intake; AN= Angus; CH= Charolais; KC= Kinsella Composite. [ab] Means different superscript letters are significantly different ($p<0.05$).

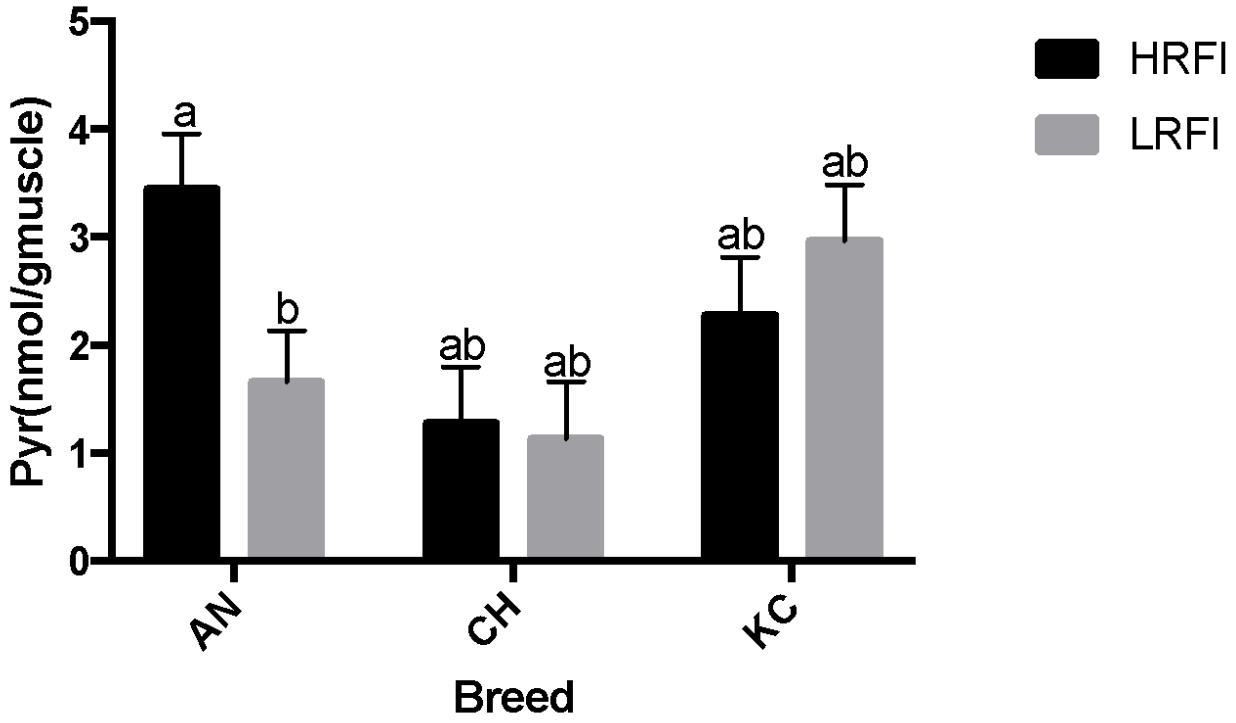

Figure 3.3 Interaction of breed and residual feed intake on pyridinoline crosslink concentration in the m. *gluteus medius*.

Error bars are standard error of the mean (SEM). LRFI= low residual feed intake; HRFI= high residual feed intake; AN= Angus; CH= Charolais; KC= Kinsella Composite. [ab] Means different superscript letters are significantly different ($p<0.05$).

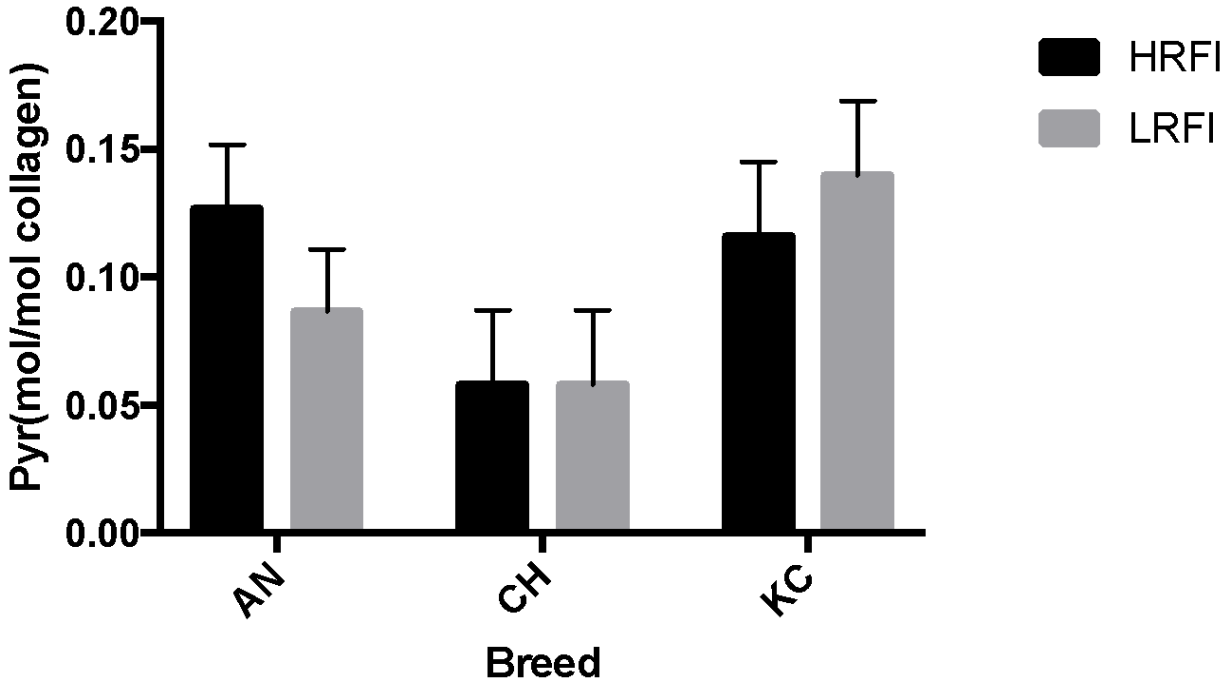

Figure 3.4 Interaction of breed and residual feed intake on pyridinoline crosslink density in the m. gluteus medius.

Error bars are standard error of the mean (SEM). LRFI= low residual feed intake; HRFI= high residual feed intake; AN= Angus; CH= Charolais; KC= Kinsella Composite. [ab] Means different superscript letters are significantly different ($p<0.05$).

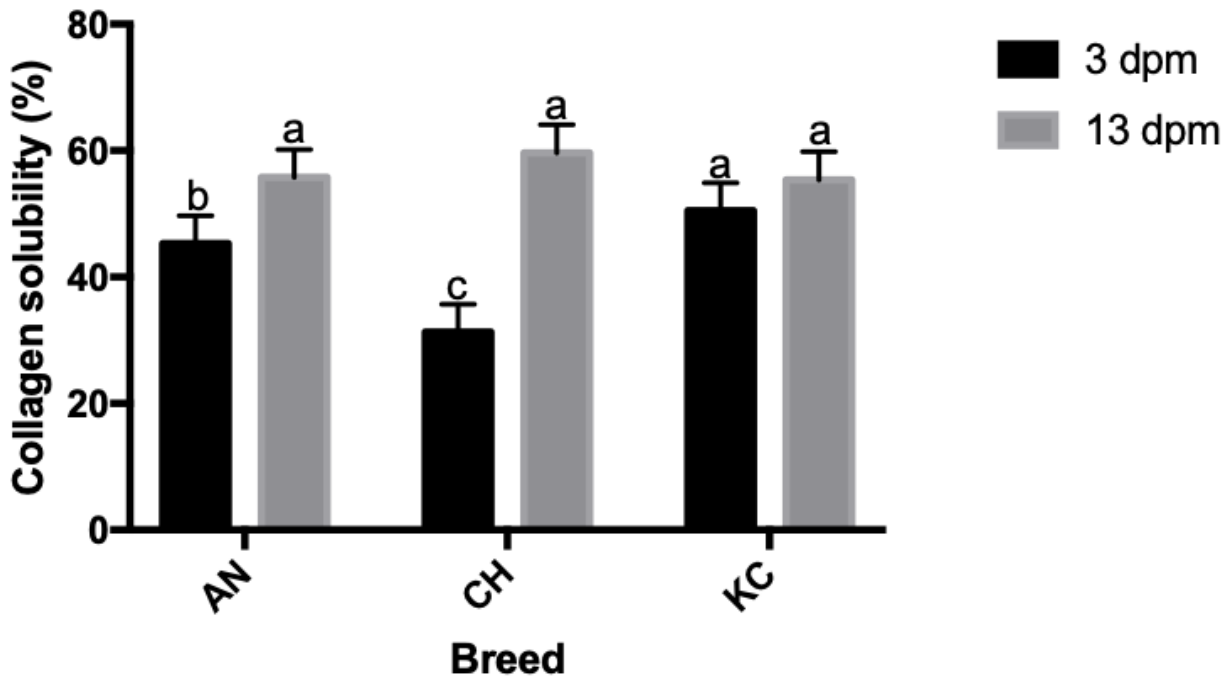

Figure 3.5 Interaction of breed and post mortem ageing on collagen solubility of the m. gluteus medius.

Error bars are standard error of the mean (SEM). 3dpm= days post-mortem; 13dpm= days post-mortem AN= Angus; CH= Charolais; KC= Kinsella Composite. [ab] Means different superscript letters are significantly different ($p<0.05$).

Chapter 4
Conclusion and Recommendations

4.1 General summary

The overall study was conducted with three breed types (Angus, Angus crossbred and Charolais), two levels of residual feed intake (RFI) (high and low) and ageing post-mortem at two different times. In Chapter 3, a subset of steers from the first study described in Chapter 2 was used to investigate if the expression of genes involved in collagen synthesis and degradation were related to collagen solubility and affected by breed as Chapter 2 indicated no effect of RFI on collagen characteristics. These studies confirmed that collagen in the *gluteus medius* was influenced by ageing post-mortem because collagen became more heat soluble with ageing. Although not investigated, these results challenge the paradigm that collagen is an intractable protein that does not change with post mortem ageing (Manka et al. 2012). These results are some of the few that clearly support the hypothesis that matrix metalloproteinases are active during the post mortem period and may be affecting collagen solubility (Sylvestre et al. 2002). The matrix metalloproteinases chosen for inclusion in the gene expression study did not show any influence of breed or RFI, nor were they related to the changes in collagen solubility. Future work should include examining the changes in collagen solubility in beef with ageing and gene expression of other MMP's that are collagenases as they are most likely the first to degrade collagen and release it from the extracellular matrix (Harris and Krane 1974).

Importantly, results indicated that low residual feed intake status in this study had a limited effect on meat quality traits and collagen characteristics. As a result, residual feed intake can be used as a selection tool by beef producers as a way to increase profits because animals that are more efficient consume less dry matter for the same weight gain. Then this characteristic can be inherited without meat quality being compromised will make it an important tool for increasing the efficiency of beef production. The effect of RFI may vary with muscle type, as RFI appears to decrease the fat content of the carcass (Basarab et al. 2003) and the distribution and deposition of fat varies by muscle (Lonergan et al. 2019). That fat is deposited

within the perimysium may also affect collagen content and beef toughness, as Roy et al. (2018) found that adipose in the perimysium was associated with decreased shear force in horse meat.

The shear force results also indicated that it is possible to use the Charolais breed without compromising the aged toughness of the GM muscle, which is the main muscle of the top sirloin, as the mean shear force value for GM from the Charolais was not different from that of the other breeds. This is useful to know for producers because this breed is characterized as a breed that increases carcass yield and potentially profits for the beef producer through yield alone. That its toughness as measured using shear force is comparable to that of Angus and Angus crossbred steers means that it can be used by beef producers without concern for an increase in beef toughness. The present study may help the beef producers have confidence in selecting steers that are more efficient, resulting in cost reduction in feed supplies and more profits. Moreover, the Charolais breed may also be used with confidence by beef producers as this breed may be used to increase carcass yield without compromising meat quality.

None of the Pearson correlations in (Chapter 2 and Chapter 3) showed correlations with shear force, with the exception of those between shear force and cooking loss and cooking time. The significance of these correlations suggests that myofibrillar proteins were determining shear force of the *gluteus medius* as no correlation was found between shear force and total collagen or collagen solubility, nor were any of the genes correlated with shear force. This thesis confirmed however that the process of ageing is a reliable method for improving beef tenderness, as shear force decreased with postmortem ageing concomitant with an increase in collagen solubility.

4.2 Further work and study limitations

The results of this study should be related to results of sensory panel evaluation of the *gluteus medius* muscle. Relating meat quality, collagen characteristics and gene expression with sensory panel results will provide a more complete understanding of what affects overall sensory perception of tenderness and how post-mortem ageing is affecting the tenderness of this muscle, because tenderness is the one attribute in which consumers are most interested as they desire reliably tender meat.

With identification of the possible relationship with genes in the m. *gluteus medius* with shear force, adding other genes like LH1, LH2a, LH2b and LH3, which are responsible for collagen crosslink formation and the

hydroxylation of the collagen telopeptide, will assist with determining if upregulation of lysyl hydroxylase is involved with preferential formation of PYR. In the second study (Chapter 3), the gene expression analysis was performed with steers selected for high and low collagen solubility and there was no significant difference in gene expression between the two populations. Future work may focus on examining the effect of selection for residual feed intake on the level of expression of primers related to calpain and calpastatin to observe if gene expression in efficient animals is correlated with meat quality characteristics rather than only assessing collagen solubility, which appeared unrelated to shear force in this muscle. Further analysis of other genes in the *gluteus medius* related to myofibrillar degradation may elucidate other mechanisms for controlling meat texture and other quality attributes. Also, proteomics characterization of these beef samples may elucidate the impact of different gene expression levels on the proteins ultimately produced and their levels of activity and function in determining beef quality and the contribution of collagen to it.

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