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Modification of steer muscle composition and meat quality through age at slaughter, hormonal implants, ractopamine hydrochloride feed supplementation, and breed crosses

by

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ABSTRACT

Management systems that enhance carcass yield can increase the efficiency and competitiveness of beef producers, but meat quality should not be negatively affected. Continental or British crossbred steers were finished to either 12 to 13 or 18 to 20 months of age and were either untreated or treated with hormonal implants, ractopamine hydrochloride, or both. Slaughtering steers at 18 to 20 month of age, using British-Continental crossbreeding, and implanting steers reduced meat tenderness but meat yield was greater than that of 12 to 13 month old steers, British-British crossbred steers, and non-implanted steers. Decreased meat tenderness was associated with increased myofibre diameter and reduced collagen solubility. Slaughtering at 18 to 20 months of age, hormonal implants, and ractopamine hydrochloride reduced meat water-holding capacity. Muscle weight appeared to be the best indicator of shear force and it may cumulatively represent complex changes related to age and growth that occur in muscle.

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LIST OF ABBREVIATIONS

α-AR	α-Adrenergic receptor
ADP	Adenosine diphosphate
ALA	Alanine
ATP	Adenosine triphosphate
β-AA	β-Adrenergic agonist
β-AR	β-Adrenergic receptor
C	Carboxy
cAMP	Cyclic adenosine monophosphate
CRA	Charolais-Red Angus
CT-SF	Connective tissue shear force
CP	Crude protein
DFD	Dark, firm, and dry
DMB	Deoxymyoglobin
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
EGTA	Ethylene glycol tetra-acetic acid
FG	Fast glycolytic
FOG	Fast oxidative glycolytic
GH	Growth hormone
GLY	Glycine
GM	M. *gluteus medius*
HAA	Hereford-Aberdeen Angus
HCl	Hydrochloric acid
HYL	Hydroxylysine
HYP	Hydroxyproline
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein

IMP	Hormonal growth implant
L*	Lightness
MF-SF	Myofibrillar shear force
MMB	Metmyoglobin
MMP	Matrix metalloproteinase
MT-MMP	Membrane type matrix metalloproteinase
mRNA	Ribonucleic acid messenger
N	Amino
NOIMP	No hormonal growth implant
NORAC	No ractopamine hydrochloride
OMB	Oxymyoglobin
PRO	Proline
PSE	Pale, soft, and exudative
RAC	Ractopamine hydrochloride
RNA	Ribonucleic acid
SDH	Succinate dehydrogenase
SEM	Standard error of the mean
SF	Shear force
SO	Slow oxidative
ST	M. *semitendinosus*
STD	Standard deviation
TIMP	Tissue inhibitors of metalloproteinase
TDN	Total digestible nutrients
WBSF	Warner-Bratzler shear force
ZH	Zilpaterol hydrochloride

Chapter 1

Literature review

1.1 INTRODUCTION

With its nutritive value, especially its high protein content, meat is an important food in human nutrition. Meat is derived from the muscles of animals killed specifically for the harvest of their muscles and livestock is now raised for this purpose in specialized production systems. In Canada, the most consumed meats are beef, pork, and chicken, whereas mutton, lamb, and turkey are marginally consumed (StatCan, 2010a). Moreover, beef was the most consumed meat in Canada in 2009 with 12.1 kg/person versus chicken (10.53 kg/person) and pork (9.66 kg/person) (StatCan, 2010a).

As the most popular meat consumed in Canada, beef is an important industry for the province of Alberta and nationally it constituted a $5.8 billion industry in 2009 (StatCan, 2010b). In Alberta, calves are born in both spring (March, April and May) and autumn (September, October, November). Spring-born calves are raised on pasture and finished on grain during the winter and sold at the market when they are approximately one year old. Fall-born calves are raised on hay and pasture, and finished on grain during their second winter. In both cases, raising cattle is a long process and generates production costs in the form of feed, yardage, bedding, and labour. A strategy used to reduce these costs is to shorten the lifetime of beef cattle by increasing their growth rate through exogenous growth promotants (CCA and BIC, 2001). Hormonal implants and β-adrenergic agonists are two common growth promotants used in feedlots. Although they improve growth rate, exogenous growth promotants can have no effect (Kerth et al., 2003; Quinn et al., 2008), can improve (Cranwell et al., 1996), or can reduce (Strydom et al., 2009) meat quality. Adverse effects on meat and consumer appreciation of it can result from the use of these substances and therefore their effects should be fully understood so that beef quality is not negatively affected.

Consumers sustain the meat industry by eating meat, thus the priority of the meat industry should be to maintain consumer eating satisfaction at a high level. Sensory properties of meat are important determinants of eating satisfaction and can be described in terms of juiciness, tenderness, springiness, flavour, and off-flavours. Although different surveys show divergent results, consumers in the survey of Huffman et al. (1996) listed tenderness as the most important factor affecting their eating satisfaction. In fact, meat tenderness has long been a concern because Bratzler (1949) referred to the mechanical determination of tenderness performed by Lehman and coworkers in 1897. In research, meat tenderness is evaluated either by a sensory panel or with a mechanical device such as the Warner-Bratzer shear force machine. Shear force is defined as the force in kg or Newtons required to shear a core of cooked meat of a definite size perpendicular to its muscle fibres. Muscle fibres and connective tissue have been shown to affect cooked meat tenderness and shear force (Crouse et al., 1991; Lepetit, 2008). The contribution of myofibrillar proteins and connective tissue to cooked meat toughness can be affected by many factors, which include the rate of animal growth (Pringle et al., 1993; Purslow, 2005), animal age (Shorthose and Harris, 1990; Wegner et al., 2000), pre-slaughter stress (Ferguson and Warner, 2008), rate of carcass chilling (Lochner et al., 1980), extent of post mortem refrigerated ageing (Devine et al., 2002; Koohmaraie, 1996; Sylvestre et al., 2002), and cooking methods (Berry, 1993). Each of these will either affect the proximate composition, biochemical, and/or physical properties of muscles and meat, which can be determined by various laboratory analyses.

Despite beef being the most widely consumed meat in Canada, the consumption of beef has declined steadily since 1985 (StatCan, 2010a). This decline coincided with the introduction of rapid growth Continental breeds in the 1960's and 1970's (CBEF, 2009; FPBQ, 2006) and the advent of hormonal growth promotants (Gould, 2000) to improve feed efficiency and carcass yield. This association highlights the disparity between efficient beef production and beef quality and indicates that the effects of beef production practices are not fully understood. For the impact of beef production practices to be understood, how these practices affect muscle and its proteins need to be considered. In order to achieve this understanding, the structure of muscle and its mechanisms of growth and how the state of the live animal affects the transformation of its muscle into meat must be considered.

1.2.1 Muscle structure

Muscles are organs and they can account for up to 40% of body mass (Akers and Denbow, 2008). Muscles act as a protein store to accommodate the energy and protein requirements of the body between meals. The composition of skeletal muscles, presented in Table 1-1, is an average because the components within muscle vary with growth and development. Water content of muscle ranges from 70 to 78%, reaching a maximum during embryonic development and then decreasing with animal age. Muscle lipid content, which ranges from 1 to 13%, is inversely proportional to the water content so tends to increase with animal age. Protein varies from 15 to 22% and decreases with fat content and age in a manner similar to that of the water content. Muscle carbohydrate is relatively consistent, varying from only 1 to 2% throughout the life of the skeletal muscle. Muscle also contains soluble non-protein substances that include minerals, vitamins, and nitrogenous substances, and these components account for approximately 4% of muscle, with their contents increasing with age (Pearson and Young, 1989).

Muscle protein is often classified into three different groups: myofibrillar, sarcoplasmic, and stromal proteins. The myofibrillar proteins are the proteins that constitute the muscle structure and include the proteins of the sarcomere, which is the basic contractile unit of muscle (Section 1.2.1.3). Sarcoplasmic proteins include more than 1000 different proteins that are found in the cytoplasm of the muscle cell, the sarcoplasm. They have a wide range of roles, usually enzymatic, such as fatty acid oxidation, glucose and glycogen metabolism, phosphorylation, electron transport, and protein synthesis and degradation (Pearson and Young, 1989). Stromal proteins of muscles include the fibrous proteins collagen and elastin. Collagen is the most important and abundant protein in this group (Sections 1.2.4 and 1.3) and is a key structural protein in the connective tissue layers that surround and permeate muscle (MacIntosh et al., 2006). The layer of connective tissue that surrounds whole muscle is referred to as the epimysium (MacIntosh et al., 2006).

1.2.1.1 Muscle fascicles

Muscles are divided into bundles called muscle fascicles, which are groups of muscle fibres held together by a connective tissue layer. In fact, two types of bundle are discernible with large fascicules encompassing small, primary fascicular bundles (Aberle et al., 2001). The tough, thick layer of connective tissue surrounding both the primary and secondary bundles is termed the perimysium and it is connected to the epimysium (MacIntosh et al., 2006). The perimysium contains blood vessels, fat, and nerves (Akers and Denbow, 2008) and its division of muscle into bundles can be discerned by the eye providing the "grain" of the muscle (Purslow, 2005). Coarse-grained muscles, meaning muscles with large fascicules, generally exhibit a greater postnatal growth than fine-grained muscles (Lawrie and Ledward, 2006). Also, muscles involved in locomotion like the m. *semitendinosus* generally appear coarser than postural muscles (Aberle et al., 2001).

1.2.1.2 Muscle cells or myofibres

Muscle fascicles consist of muscle fibres, also known as muscle cells or myofibres, which are the fundamental organizational units of muscles. Each muscle fibre in turn is encased in the endomysium, a connective tissue layer associated with the muscle cell membrane (Akers and Denbow, 2008). The muscle cell is non-mitotic, multinucleated, and comes from the myoblast cells (MacIntosh et al., 2006). During prenatal life, myoblast cells fuse to form myofibres that can reach 10 to 50 µm in width and 1 to 40 mm in length (Lodish et al., 2005). The main cellular constituents of muscle fibres are myofibrils, nuclei, and mitochondria which are bathed in the sarcoplasm (Akers and Denbow, 2008). The cellular membrane of a muscle fibre is the sarcolemma and underneath it are the muscle cell nuclei, which can number in the hundreds. The extracellular matrix of muscle fibres is the basement membrane, and it consists of two layers, the basal lamina and the reticular lamina (Sanes, 2003), which are located between the sarcolemma and the endomysium. The basal lamina is synthesized and secreted by the myofibres to form the extracellular matrix, which is composed of collagen type IV and glycoproteins (association of a protein and one or more carbohydrate groups) (Huijing, 1999).

Myofibrils are the functional subunits of muscle fibres (Frandson and Spurgeon, 1992) and consist of myofilaments, which are composed of thin protein filaments (actin) and thick protein filaments (myosin). These myofilaments give a striated appearance to the myofibrils by their division into sarcomeres, which have alternating dark and light bands (Pearson and Young, 1989). There are about 1500 thick filaments and 3000 thin filaments in one myofibril (Frandson and Spurgeon, 1992) and about 1000 to more than 2000 myofibrils per fibre of width 50 μm (Aberle et al., 2001).

Satellite cells are an important constituent of muscle fibres. They come from the myoblast cells; however, they are not fused with the muscle fibres because they are located between the basement membrane and the sarcolemma in the endomysium layer (Hossner, 2005) with capillaries and nerves (Akers and Denbow, 2008). Satellite cells are mononucleated stem cells that provide new myonuclei and help regenerate muscle fibres when the fibres are damaged (Pearson and Young, 1989). They do this by enlarging themselves and then dividing into mother and daughter cells rather than by fusing with damaged cells. The daughter cell then differentiates, which is signalled by the production of myofilaments, so that it is no longer able to divide and it becomes a new muscle cell (Pearson and Young, 1989).

1.2.1.3 Sarcomeres

Sarcomeres are the basic contractile units of muscles and are present in series of thousands in a myofibril (Pearson and Young, 1989). The alternating dark and light bands that are evident when muscle is viewed microscopically are the result of the overlapping thin and thick filaments within the sarcomeres. Sarcomeres range from 1.3 to 3.0 μm long, with the thin and thick filaments measuring about 1 μm and 1.6 μm in length, and 5 to 6 nm and 10 to 12 nm in diameter, respectively (Akers and Denbow, 2008). The thin filaments are anchored to Z-discs or Z-bands at each end of the sarcomere (Figure 1-1), which define the length of each sarcomere. The light band, also called the I-band (I = isotropic), is formed solely of thin filaments that are attached to the Z-bands, or Z-discs. A Z-band forms a dark line in the middle of the I-band (Pearson and Young, 1989). There is another dark band, the A-band (A = anisotropic), which consists of thick and thin filaments; however, the thin filaments do not cover the entire length of the thick filaments and this creates a light H-band in the center of the A-band where the free ends of thin

filaments are located. In this H-band is the M-line, which consists of five protein lines known as M-filaments that stabilize the thick filaments (MacIntosh et al., 2006). The structure of the sarcomere allows for the thin and thick filaments to slide past each other during extension and contraction, allowing muscle movement. Regardless of the length of the sarcomere, there is always one-half I-band, an A-band, and another one-half I-band in each sarcomere.

1.2.1.4 Myofibrillar proteins

The thick and thin filaments and the Z-bands are the three major structures that facilitate contractive movement by the sarcomeres. Within those three structures are contractile, regulatory and cytoskeletal proteins, which constitute the myofibrillar proteins (Pearson and Young, 1989). These proteins account for between 55% and 65% of the proteins found in myofibres (Hossner, 2005) and they occupy approximately 25% of the cellular space (Pearson and Young, 1989).

Contractile proteins include actin and myosin, the two main myofibrillar proteins active in muscle contractions (Pearson and Young, 1989). Myosin and actin represent 60% and 20% of the total myofibrillar protein content, respectively (Obinata et al., 1981) and are soluble in concentrated salt solution (Lawrie and Ledward, 2006). Myosin is a molecule formed from two polypeptide chains linked in a coiled-coil α-helix structure. The coiled-coil helix constitutes the myosin tail and is referred to as light meromyosin. Each α-helical chain continues individually and ends in a mobile head, giving two heavy meromyosin heads to each myosin molecule that are commonly termed the myosin heavy chains. Then, each head splits into two protein subunits, which are the myosin light chains. A myosin filament consists of approximately 150 myosin molecules (Wehner and Gehring, 1999). Actin can exist in two forms: as F-actin (filamentous actin) and G-actin (globular-actin) (MacIntosh et al., 2006) with F-actin the polymerized form of G-actin (Akers and Denbow, 2008). In muscle, an actin filament is an F-actin double helix.

The troponins and tropomyosin are regulatory myofibrillar proteins found in the thin filament. The troponins are present every seventh G-actin molecule (Wehner and Gehring, 1999) and are a complex of three globular proteins: troponin I, troponin T, and

troponin C (Pearson and Young, 1989). Tropomyosin coils around the F-actin double helix in the same direction as the actin (Wehner and Gehring, 1999).

The cytoskeletal protein family also includes several proteins that are constituents of sarcomeres and take part in the support and alignment of the thin and thick filaments. Located in the I-bands, nebulin is a scaffold protein for the thin filament and binds thin filaments to Z-discs (Aberle et al., 2001). The M-lines contain myomesin, M-protein, and skelemin, proteins that help connect and align adjacent thick filaments (Aberle et al., 2001). C-protein and H-protein are present in the region of the H-band, with the former encircling thick filaments (Aberle et al., 2001). Alpha-actinin and Cap Z constitute the Z-disks and desmin, filamin, paranemin, and synemin are found in the thin filaments and bind together adjacent Z-discs to give the lateral arrangement of adjacent myofibrils (Aberle et al., 2001). Titin, also known as connectin, is found throughout sarcomeres and attaches thick filaments to M-lines and Z-discs (Akers and Denbow, 2008). Other cytoskeletal proteins such as dystrophin, talin, and vinculin are present in very small amounts in sarcomeres (Aberle et al., 2001) and appear to be connector proteins.

1.2.2 Classification of fibres

Muscle and muscle fibre classifications are closely related because muscle fibres are the motor units of muscles and collectively define the fibre type of the muscle. Skeletal muscle fibres can be classified according to several systems and most commonly have been differentiated on their colour, metabolism, and protein isoform (Akers and Denbow, 2008; Pearson and Young, 1989). Protein isoforms are different forms of protein from the same protein (Lodish et al., 2005). Fibres can be classified as red, white, or intermediate according to their colour and dominant energy metabolism. Red fibres use an oxidative metabolism that requires oxygen and encompasses the Krebs cycle. The molecule binding oxygen and needed for its functioning is myoglobin, a red pigment protein present in great proportion in red fibres. Myoglobin, consisting of the protein globin, and heme, a non-protein domain containing iron, represents about 80 to 90% of the muscle pigments (Lawrie and Ledward, 2006). Red fibres also have a high number of capillaries for a concentrated blood supply to transport oxygen to the mitochondria where the aerobic Krebs cycle occurs. White fibres use primarily the glycolytic metabolism, which obtains adenosine triphosphate (ATP) from glucose anaerobically as energy for

cellular functions in anaerobic conditions such as exercise. As a result, white muscle fibres require less oxygen than red muscle fibres and have a low content of myoglobin. White fibres are termed so because myoglobin, that imparts a red colour to muscle, is lacking in these fibres. Intermediate fibres use both metabolisms, have a high content of myoglobin, mitochondria, and capillaries, and a glycogen content that is higher than in red but lower than in white fibres (Akers and Denbow, 2008).

When classified based upon the dominant cellular metabolism, fibres are typed by their myofibrillar ATPase activity and succinate dehydrogenase (SDH) staining. SDH is used to assess the concentration of mitochondria because this enzyme is located in their inner membrane. Quantification of SDH indicates the extent of oxidative metabolism in the cell or muscle. By differentiating muscle fibre types using ATPase and SDH staining, fibre types can be identified as β-red, α-red, and α-white. Staining for SDH will darken the areas of the cell that have mitochondria and so will darken only the edges of α-red fibres but will stain β-red fibres throughout. The myofibrillar ATPase activity refers to the speed of contraction, engendered by the speed of ATP hydrolysis by the myosin ATPase. α-Red and α-white fibres have high myofibrillar ATPase activity, unlike β-red fibres (Pearson and Young, 1989).

When using the speed of contraction and the oxidative and glycolytic capacities to classify fibres, fibres can be described as slow oxidative (SO) or type I, fast oxidative glycolytic (FOG) or type IIA, and fast glycolytic (FG) or type IIB. The speed of twitching used in this system is based on the speed of myosin ATPase by the myosin heavy chains similar to that described in the previous classification system. In fact, according to the myosin heavy chains, there is another type of fibre, designated as X, resulting in four types of fibres: types I, IIA, IIX, and IIB (Lefaucheur, 2010). Fibres can change type, with the transition between fibre type usually following the pattern I↔IIA↔IIX↔IIB (Lefaucheur and Gerrard, 2000), but little is known about type IIX fibres because they are a relatively new addition to fibre classification. The acid/base lability of fibres is used during staining to distinguish the myosin isoforms. Resistance to fatigue is related to an oxidative muscle metabolism, which can provide energy for a prolonged period of exercise while a glycolytic metabolism can only sustain short term energy needs. SO fibres are thus slow twitching, fatigue resistant, have an oxidative metabolism (Pearson and Young, 1989), and are acid labile (Brooke and Kaiser, 1970). FOG fibres are fast

twitching, moderately fatigue resistant, neither acid labile nor base labile, and have a mix of oxidative and glycolytic metabolisms. Finally, FG fibres are fast twitching, fatigue sensitive, use the glycolytic metabolism (Pearson and Young, 1989), and are base labile (Brooke and Kaiser, 1970). There are similarities between the various fibre classifications, with SO fibres equivalent to red fibres, FOG representative of intermediate fibres, and FG similar to white fibres (Chang, 2007).

1.2.3 Classification of muscles

Within a muscle, a mixture of red and white fibres is found and gives rise to muscles being classified as white, intermediate, or red based upon how much of one fibre type they have or their degree of redness or whiteness (Pearson and Young, 1989). Furthermore, in this classification of muscles, the *m. semitendinosus* (ST) and the *m. gluteus medius* (GM) are classified as white muscles. Indeed, according to their fibre type composition the ST had 49.7% of white fibres and the GM contained 55.6% of white fibres (Kirchofer et al 2002).

1.2.4 Connective tissue as a part of muscle

Connective tissue consists of a hydrated polysaccharide gel that is the ground substance, cells, and protein fibres (Huijing, 1999). Glycosaminoglycans bind to protein to form proteoglycans in the ground substance. Fibroblasts are embedded in the matrix formed by the protein fibres and the ground substance (Akers and Denbow, 2008) and synthesize the components of the extracellular matrix (Lodish et al., 2005). What differs between types of connective tissues are the fibre number and arrangement between cells. Connective tissues are mostly found in muscles as part of the epimysium, perimysium, and endomysium, the two latter layers being the intramuscular connective tissue. There are also other structures which contain connective tissue such as tendons, ligaments, and blood vessel walls (Bailey and Light, 1989).

The stromal proteins forming connective tissue include the fibrous protein collagen, amounting to 25% of the total body proteins (Wehner and Gehring, 1999). Collagen is mostly located in skin, bones, tendons, and arterial walls. In beef muscles, based on the dry weight, total collagen varies from 1% to 15% depending on the location

and function in the body (Purslow, 2005). Fibroblasts synthesize collagen in muscles and collagen is a major constituent of the epimysium, perimysium, and endomysium (Pearson and Young, 1989). Goldberg and Green (1968) demonstrated that other cell lineages can synthesise procollagen molecules as well, although the secretion of collagen is less important in other cells than it is in fibroblasts.

The other main protein of connective tissue fibres is elastin. Elastic fibres consist of two proteins: elastin, which is an amorphous component, and the microfibrillar component. Like collagen, elastin is classified as a fibrous protein and is secreted by the fibroblasts (Pearson and Young, 1989). This fibrous protein is formed intracellularly, although it is cross-linked extracellularly (Brown-Augsburger et al., 1995). Elastin muscle content is generally much lower than collagen content; however, the content of elastin in the ST is relatively high compared to other muscles (Bendall, 1967). Indeed, according to this author, the ST muscle contains 3.1% of collagen expressed as a dry muscle weight and 1.82% of elastin while the GM contains 1.6% of collagen and 0.18% of elastin. The difference is considerable because elastin represented 10% of the connective tissue in the latter muscle, but 37% in the former. Bendall (1967) also mentioned that the contribution of elastin to cooked meat toughness can be ignored in the GM while in the ST the effect of elastin on cooked beef toughness is similar to denatured collagen. As demonstrated by Cross et al. (1973), elastin was not correlated with cooked beef meat shear force even in the ST. Shear force (SF) is an estimate of cooked meat toughness and is the force in kg required to shear a core from a cooked steak.

1.3 CONNECTIVE TISSUE STRUCTURE

1.3.1 Collagen classification

Approximately 28 types of collagen have been discovered to date (Khoshnoodi et al., 2006). They are grouped according to their macromolecular structure and α-chains as shown in Table 1-2. Basically, three α-chains compose a collagen molecule, although there are exceptions, and each α-chain is identified using ‘αn(N)’ terminology where ‘n’ is the number of the α-chain and ‘N’ is the collagen type. A collagen molecule is termed homotrimeric if its three α-chains are identical and heterotypic if they are not (Hulmes,

2008). Some collagens have only recently been discovered, thus information is incomplete or not available.

The collagen molecule family most pertinent to the eating quality of meat is the fibril-forming family because, as the classification name suggests, collagen types from this family form collagen fibrils. Of the fibril-forming collagens found in skeletal muscles, types I and III collagen are the major types (Lepetit, 2007). The endomysium of muscle cells is mostly formed by types I, III, IV, and V collagens (Kovanen, 2002) and the collagen fibrils of these collagen types form fine and slightly wavy fibrils (Lepetit, 2008). The perimysium consists mostly of types I and III collagen (Kovanen, 2002) and represents about 90% of the connective tissues in muscles, although this is variable from muscle to muscle (McCormick, 1999). Unlike the fine wavy fibrils of the endomysium, the fibrils present in the perimysium are thick and highly wavy (Lepetit, 2008). The epimysium consists mostly of type I collagen that forms parallel fibres in this tissue (Kovanen, 2002) and makes this layer thick, tough, poorly soluble, and shear-resistant (McCormick, 1999). Collagens V and XI can be considered a sub-family of the fibril-forming collagens because their α-chains can crosslink together to form a type V/XI collagen fibril (Eyre and Wu, 2005).

1.3.2 Collagen amino acid composition

Five amino acids dominate the primary structure of collagen, glycine (GLY), proline (PRO), alanine (ALA), hydroxyproline (HYP), and hydroxylysine (HYL). They constitute 33, 12, 11, 10, and 1% respectively of the amino acid composition of fibrils-forming collagens (Pearson and Young, 1989). Individually, tyrosine, histidine, methionine, and cysteine account for less than 1% of all amino acids within a collagen α-chain (Pearson and Young, 1989). GLY, PRO, and HYP are present in large concentrations because they support the formation of each collagen chain in an α-helix and allow the formation of the collagen molecule triple helix (Voet and Voet, 2005).

There are two frequent amino acid sequences that occupy 21% of the total amino acid sequence: GLY-PRO-Y and GLY-X-HYP, where X and Y can be any amino acids (Pearson and Young, 1989). The sequence GLY-X-Y is repeated five or six times, then an amino acid polar sequence occurs (Pearson and Young, 1989). The unusual amino acid

HYP is unique to collagen and is used for the collagen content determination (Ottani et al., 2002). This amino acid also stabilizes the collagen triple helix by hydrogen bonds (Voet and Voet, 2005). HYL, another unusual amino acid present in collagen, is involved in intermolecular cross-linking between and within collagen molecules and links carbohydrate groups to collagen as well (Pearson and Young, 1989).

1.3.3 Collagen formation

The primary structure of collagen is determined by genes that code for an amino acid sequence that will form an α-chain. Amino acids are synthesized as polypeptides by the polysome, which is the assembly of messenger ribonucleic acid (mRNA) and a ribosome (Bailey and Light, 1989). The secreted polypeptide goes directly into the rough endoplasmic reticulum of the fibroblast (Pearson and Young, 1989). After its secretion, an α-chain becomes folded in a left-handed twisted-geometry structure, the secondary structure of collagen (Voet and Voet, 2005). This special geometry is possible because of the C-H fixed angle of the peptidyl-proline or peptidyl-hydroxyproline bonds. The whole polypeptide of α1(I) collagen molecules contains about 1042 amino acids; however, only 1011 amino acids are involved in the α-chain helix (Voet and Voet, 2005). The remaining amino acids present in the amino and carboxyl terminals of α-chains are not twisted, thus are not in the triple helix structure. This happens because GLY is not present every third residue in the sequence to promote the twisted geometry. Instead, lysine is present in higher amounts than in the helix and it serves to facilitate cross-linking between collagen molecules (Pearson and Young, 1989).

HYP and HYL amino acids are synthesized before the assembly of a procollagen peptide. Immediately following translation of the mRNA in the ribosome, PRO is hydroxylated by the prolyl hydroxylase enzyme to form HYP, and the same process occurs with lysine and the lysyl hydroxylase enzyme to form HYL (Bailey and Light, 1989). These hydroxylations are required for the subsequent formation of non-covalent bonds within the procollagen helix (Bailey and Light, 1989). The enzymes implicated in this process require ascorbic acid (vitamin C), oxygen, ferrous iron, and α-ketoglutarate to be active (Pearson and Young, 1989).

Following the formation of the α-chains, three α-chains combine together and form a right-handed triple helix with staggered chains, or procollagen. Procollagen, the tertiary structure of collagen, is termed so because of the additional amino acid regions found on its carboxy terminal. In its center are the H atoms side chains of GLY because it is the only molecule small enough to go through the center of the triple helix (Voet and Voet, 2005). These H atoms link the GLY amino NH peptide bond to the PRO oxygen of a peptide carbonyl (C=O), forming a hydrogen bond and many of them tightly bind the three coiled subunits together (Voet and Voet, 2005). At the carboxy (C) and amino (N) propeptide terminals, disulfide bonds are formed to keep the α-chains in a proper alignment (Bailey and Light, 1989). The left-handed twisted-geometry of the collagen polypeptide and the right-handed triple helix of procollagen confer a strong resistance to the triple-helix (Voet and Voet, 2005).

After its release from the rough endoplasmic reticulum, procollagen molecules are moved to the Golgi apparatus to be assembled in a lateral arrangement (Lodish et al., 2005) as shown in Figure 1-2. Then, molecules are secreted towards the cell surface in a specialized structure called the microtubular microfilament system (Bailey and Light, 1989). Procollagen proteinases are specialized enzymes that remove about 20 residues of non-helical structure at both the N and C propeptide terminals from the type I, II, and III procollagen molecules after their secretion in the extracellular space (Hulmes, 2008). After the removal of the propeptides, procollagens lose their lateral arrangements (Lodish et al., 2005).

Many procollagen molecules gather together spontaneously (Voet and Voet, 2005) to form a collagen fibril of 50 to 200 nm in diameter (Lodish et al., 2005). Collagens are assembled in lateral head-to-tail arrangements, as shown in Figure 1-3, with hydrophobic and electrostatic interactions (McCormick, 1999). Between two adjacent collagen molecules of a single row is a space of about 67 nm (Bailey and Light, 1989) and rows of collagen molecules are staggered between them, causing an overlapping of adjacent rows. This structure gives a striated appearance to collagen fibrils. There are also disaccharides in collagen fibrils formed by galactose and glucose that are enzymatically linked to HYL amino acids; however, the functions of these disaccharides are not as yet known (Voet and Voet, 2005).

The special striated arrangement of collagen fibrils requires cross-links that can be intramolecular or intermolecular. Cross-links confer to collagen a high stability, a reduction in solubility, and an increase in tensile strength (Kovanen, 2002). Newly synthesized collagen fibrils associate spontaneously to first form fascicles 50 to 300 µm wide and then collagen fibres 100 to 500 µm long (Fratzl, 2008). The assembly of collagen fibrils is facilitated by small leucine-rich repeat proteoglycans (Graham et al., 2000) like decorin, fibromodulin and lumican (Geng et al., 2006). Collagen fibres are the quaternary structure of collagen and they form wide structures such as the perimysium, the epimysium, and the endomysium.

1.3.4 Collagen cross-linking

As mentioned before, cross-links in collagen can be intramolecular or intermolecular. The latter type of cross-link is the one responsible for the high strength of collagen quaternary structures (Myllyharju and Kivirikko, 2004). Nonetheless, cross-links seem more tissue-specific than collagen type specific (Pearson and Young, 1989) suggesting that cross-link type is determined by function rather than protein form.

1.3.4.1 Intermolecular cross-links in fibril-forming collagens

Intermolecular cross-linking can occur between different collagen types. For instance, cross-links can form between collagen types I and III, types I and II; types I and V with XI; or types II and V with XI (Lodish et al., 2005).

The formation of intermolecular cross-links is catalyzed by the lysyl oxidase enzyme, which contains one copper ion (Rucker et al., 1998). This reaction occurs specifically on lysine and HYL side chains, giving two different pathways for the formation of aldehydes. The lysine aldehyde-initiated pathway occurs in the skin, whereas the hydroxylysine aldehyde-initiated pathway occurs in bones, cartilage, and muscles (Eyre and Wu, 2005). Only the latter pathway will be described further as it is the one that occurs in muscle tissue.

Divalent cross-links link two collagen molecules (Lepetit, 2008). There are four cross-linking sites, of which two sites are on the N terminal, in the telopeptide and the

helical regions, and two sites in the C terminal, in the telopeptide and the helical regions as well (McCormick, 1999) (Figure 1-4). The formation of intermolecular cross-links by the hydroxylysine cross-linking pathway starts with the telopeptide lysine and hydroxylysine residues that are converted by the lysyl oxidase enzyme, using Cu^{2+} and O_2, into a telopeptide allysine and a telopeptide hydroxyallysine. Then, divalent cross-links are formed by the reaction of the telopeptide allysine with the hydroxylysine side chains or the telopeptide hydroxyallysine with the lysine and hydroxyallysine side chains. These reactions produce aldimines and keto-amines that include hydroxyl-lysinonorleucine, hydroxyl-lysinoketonorleucine, and lysinoketonorleucine (Eyre and Wu, 2005).

Products derived from the divalent cross-linking react together to form mature trivalent cross-links that bind three collagen molecules (Lepetit, 2008), with the ability of collagen molecule to slide (McCormick, 1999). The reaction between the hydroxyl-lysinonorleucine and hydroxyl-lysinoketonorleucine cross-links form hydroxyl-lysylpyrrole, commonly referred to as Ehrlich Chromogen (one of the two names used to term a trivalent cross-link (Lepetit, 2008)) because it reacts to form a chromogen with the dye *p*-dimethylaminobenzaldehyde. The hydroxyl-lysinonorleucine cross-link can also form lysyl-pyrrole, which is another Ehrlich Chromogen. The hydroxyl-lysinoketonorleucine and lysinoketonorleucine cross-links react to form lysylpyridinoline, whereas two hydroxyl-lysinoketonorleucine cross-links form hydroxylysylpyridinoline (Figure 1-5) or pyridinoline (the second name of a trivalent cross-link (Lepetit, 2008)) (Eyre and Wu, 2005). This latter cross-link is the most frequent trivalent cross-link (McCormick, 1999).

1.3.4.2 Intramolecular cross-links

Intramolecular cross-links are possible; in fact they are dimers that originated from the allysine cross-linking pathway. This reaction occurs in the non-helical α-chain ends of collagen molecules and starts as soon as procollagens are formed and cross into the rough endoplasmic reticulum (Pearson and Young, 1989). At this point, a combination of two allysine residues located at the N terminals of two different α-chains within the same collagen molecule will form an aldol condensation product, producing a dimer (Ricard-Blum and Ville, 1989). The tensile strength of collagen is due to the intermolecular cross-

links and not the intramolecular cross-links (Davison and Brennan, 1983); therefore, intramolecular cross-links will not be further discussed.

1.3.5 Collagen turnover

The rate of collagen turnover varies considerably between different tissues as well as within a tissue depending on the maturity of the collagen. Collagen is mostly synthesized in newly formed tissues, thus immature cross-links are usually present in high concentrations in the tissues of young animals, whereas trivalent cross-links are mostly present in the tissues of mature animals (Pearson and Young, 1989). A slow connective tissue turnover gives cross-links the time to form and then to change from a divalent to a trivalent and potentially a tetravalent cross-link (Purslow, 2005) although not all collagen types form trivalent and tetravalent cross-links (Eyre and Wu, 2005). Trivalent cross-links are heat stable, whereas divalent cross-links are not (Lepetit, 2008). So the decrease in collagen heat solubility with age (Gerrard et al., 1987; Žgur et al., 2003) due to an increased number of trivalent cross-links will toughen cooked meat (Bailey and Light, 1989). The extent of the cross-link contribution to beef meat toughness is, however, muscle dependant (McCormick, 1999).

Cross-links of mature collagens make collagen more difficult to degrade than immature collagen (Kovanen, 2002). Collagen fibres are very resistant to enzymatic degradation because of their triple-helical structure but there are still enzymes able to degrade collagen and they are referred to as matrix metalloproteinases (MMP). MMP are zinc-dependant endopeptidases (Kovanen, 2002) that include collagenases, stromelysins, and gelatinases (Sylvestre et al., 2002). Collagen degradation generates pyridinoline cross-link residues that cannot be reutilized to synthesise other proteins (Eyre et al., 2008). These residues are excreted in urine, providing a way to estimate collagen turnover in the whole body.

Twenty-five MMP have been identified to date (Lauer-Fields et al., 2002) and they are collagen type-specific. For instance, MMP-1, MMP-8, and MMP-13 degrade types I and III collagen, whereas type IV collagen is degraded by MMP-2 and MMP-9. MMP activity is inhibited by tissue inhibitors of metalloproteinases (TIMP) and encouraged by the membrane type MMP. There are four types of TIMP, TIMP-1 to -4, that are known to

inhibit MMP activity, whereas there are two membrane types MMP (MT1-MMP and MT2-MMP), which activate MMP (Kovanen, 2002). Fibroblasts secrete both MMP and TIMP (Purslow, 2005). Small leucine-rich repeat proteoglycans such as decorin, fibromodulin, and lumican can reduce the susceptibility of collagen cleavage by the MMP (Geng et al., 2006) mostly likely through steric hindrance.

1.3.6 Factors affecting muscle collagen content

Collagen content varies greatly between muscles as shown by Bendall (1967) who quantified the amount of collagen of several muscles on a dry weight basis. This author found that some muscles have a very low collagen content, like the GM (1.6%), m. *adductor* (1.7%), m. *pectineus* (1.9%), and m. *quadriceps rectus femoris* (1.9%), whereas others have a high collagen content like the m. *sternomandibularis* (7.3%), m. *panniculus* (9.7%), and m. *peronaeus tertius* (15.1%). These differences among muscles are reflected in the cooked muscle SF with different degrees of tenderness such as 'slightly tough' for the ST and 'slightly tender' for the GM (Aberle et al., 2001). The amounts of total and soluble collagen are also correlated with the tenderness of raw muscles (Torrescano et al., 2003). Functions of muscle appear to affect collagen content because Prost et al. (1975) noted that muscles of the forequarter were ranked as having higher connective tissue content than those of the hindquarter. This was further explained by the forelimb muscles which are connected to the body through muscular attachments, unlike the hindlimb muscles which are attached by bones. Thus, muscle collagen contents can greatly differ and these differences should be taken into account when comparing results between muscles.

Collagen content not only varies among muscles but also within a muscle due to biochemical transmission of forces necessary for locomotion. The m. *trapezius thoracis* has a thick and a thin part, which contain 4.0% and 8.6% of collagen of the dry muscle weight, respectively (Bendall, 1967). Moreover, the anterior end of the m. *pectoralis superficialis* has 4.0% of collagen but the posterior end has 7.2%. Once again, differences in SF can be observed at different location in the muscle. A study conducted by Shackelford et al. (1997) showed that Warner-Bratzler shear force (WBSF) significantly increased from 3.87 to 5.07 kg starting from the distal end and ending at the proximal end of the ST with a mean SF of 4.05 kg.

Finally, collagen muscle content is, to a certain extent, inversely proportional to age. Young cattle generally have more collagen per gram of wet muscle tissue than their mature peers, but the quantity appears to stabilize with age (Dikeman et al., 1986). Hence, collagen content diminishes during growth most likely because it is diluted by the increase in the size of muscles through the addition of proteins and fat (Gerrard et al., 1987).

1.4 PRINCIPLES OF MUSCLE GROWTH

1.4.1 Postnatal muscle growth

Postnatally, muscles grow by hypertrophy, which is an increase in size of muscle cells through the accumulation of myofibrillar proteins, rather than by hyperplasia, which is an increase in size due to an increase in muscle cell number (Rehfeldt et al., 2004). Muscle fibre number in the bovine is set at birth, with hyperplasia continuing after birth only in the pig (Frandson and Spurgeon, 1992). The hypertrophy potential of a muscle is influenced by the number of muscle fibres, however, and this number is determined by hyperplasia during embryonic life (Hossner, 2005). The muscle fibre number and the muscle fibre size are inversely proportional because, as the number of muscle fibres increases, the growth rate of an individual muscle fibre decreases (Rehfeldt et al., 2004). As a result, the potential for postnatal growth and mature muscle mass is seriously influenced and limited by the number and size of the myofibres formed prenatally (Lawrence and Fowler, 2002).

Along with the intracellular deposition of myofibrillar proteins that occurs during muscle hypertrophy, muscle nuclei are added to the growing muscle fibres by satellite cells. Satellite cells are stem cells that slowly proliferate during postnatal growth through mitotic divisions; therefore, the muscle deoxyribonucleic acid (DNA) amount increases. These newly formed satellite cells add DNA to muscle fibres by fusing with them (Pearson and Young, 1989), consequently up to 90% of the myofibre DNA is accumulated postnatally (Hossner, 2005). When hypertrophy ceases as animals mature the number of satellite cells as a percentage of total nuclei present in mature muscle fibres decreases and remains at about 1% to 10% (Hossner, 2005).

Growth rate is muscle dependent and muscles can be divided into six classes based on their growth rate at different stages of growth (Berg and Butterfield, 1976). Muscles of the *high impetus* class are muscles growing faster than muscles from the *average impetus* class whereas *low impetus* muscles grow slower than *average impetus* muscles. *High-average impetus* muscles, like the ST and GM, grow faster early in the animal lifetime, but follow the average growth rate later during growth. Growth rate of *average-high impetus* muscles is average in early life, but is faster than the average growth rate in later phases of growth. The last classification, *low-average impetus*, represents muscles with a slow growth rate in the first phase of growth and an average growth rate later during life.

1.4.2 Postnatal muscle cell hypertrophy

During growth, muscle fibres are submitted to longitudinal stretch from the elongation of bones and respond to this stimulus by lengthening. In concomitance with this longitudinal growth, muscle fibres enlarge by adding new mitochondria, nuclei, and proteins to myofibrils, the most important being actin and myosin (MacIntosh et al., 2006).

The lengthening of myofibres is achieved through the increase in both the number and length of sarcomeres although the increase in sarcomere length is modest (Young, 1974), accounting for 25% of the total myofibrillar lengthening in mice (Goldspink, 1968). The sarcomere lengthening is an elongation of sarcomeres through a reduction of the overlapping of the I-band and A-band (Goldspink, 1968). The increase in sarcomere number is made by the addition of new sarcomeres at the end of myofibrils. As a result, sarcomeres are shorter toward the end of myofibrils than they are in the middle of myofibril and their lengths within young muscles vary more than within mature muscles (Goldspink, 1968).

When muscle fibres enlarge by adding new mitochondria, nuclei, and proteins to myofibres, they reach a critical point size at which they split longitudinally. Although splitting of muscle fibres is thought to happen more than four times during the animal's life, the split does not occur on all the myofibres, only on less than 10% (Goldspink, 1970). There are two different phases of muscle fibres: the small phase fibres and the large phase fibres. The former have a diameter of about 23 µm, whereas the latter reach a

diameter of 40 µm. The estimated minimum time to growth from a small to a large phase fibre is one to two days in mice (Goldspink, 1965). A rip within the midsection of the Z-bands is thought to cause the longitudinal split of myofibrils (Goldspink, 1970). This rip can be made by an oblique pull of actin filaments on the Z-disc when tension is developed between two adjacent sarcomeres (Goldspink, 1971).

1.4.3 Protein turnover

Protein turnover refers to the balance between protein synthesis and degradation. Although different rates can be observed for each, these two phenomena are always active resulting in continuous skeletal muscle protein turnover (Buttery and Vernon, 1980). Protein turnover occurs for many reasons including harvesting of amino acids for glucose or remodelling of muscle during growth. For growth to occur, the rate of protein synthesis must be greater than the rate of protein degradation. There are different scenarios by which hypertrophy is possible (Koohmaraie et al., 2002):

- Protein synthesis increases whereas protein degradation decreases or remains stable;
- Protein synthesis increases or remains stable whereas protein degradation decreases;
- Protein synthesis and degradation both increase, but increase in synthesis is greater than increase in degradation;
- Protein synthesis and degradation both decrease, but degradation decreases more markedly than synthesis.

The processes of protein synthesis and degradation are more active in young animals than in mature animals. In a youthful animal, protein synthesis is higher than degradation leading to muscle growth. The protein turnover rate declines rapidly as the animal ages, and growth eventually stops when protein synthesis is equal to protein degradation. Protein turnover varies also between muscles and a potential explanation for this phenomenon is the proportion of muscle fibre types within muscles.

Protein degradation is driven by enzymes that cleave proteins, called proteases or proteolytic enzymes, which participate in the dismantling of protein quaternary structure. Calpains are proteases that take part in the synthesis-degradation balance. The calpains

are present in two forms, m-calpain and μ-calpain, (Hossner, 2005) and are ubiquitous (Houbak et al., 2008). The calpains are activated by specific Ca^{2+} intracellular concentrations, thus they are calcium-dependant. The names of m-calpain and μ-calpain refer to the amount of calcium required for their activation. M-calpain requires millimolar concentration of Ca^{2+}, about 1 to 2 mM, whereas μ-calpain requires micromolar concentration of Ca^{2+}, about 50 to 100 μM (Etherington, 1984). Calpains degrade proteins into large polypeptide chains, which are then further degraded into amino acids by other proteases such as cathepsins and proteasomes. Calpain levels in muscles are relatively consistent; however, levels of calpastatin, the protein inactivating calpains, vary with physiological states (Hossner, 2005).

Cathepsins, also known as lysosomal proteases because they are contained in lysosomes, are other proteases acting in the synthesis-degradation balance. The cathepsins A, C, and D act as endopetidases cleaving peptides at their peptide bonds, whereas the cathepsins L and B are endo- and exopeptidases (Hopkins and Taylor, 2004). Cystatin, located in the muscle cell sarcoplasm, inhibits the activity of cathepsins (Hopkins and Taylor, 2004).

Degradation of proteins by proteasomes is very important in skeletal muscles (Los and Haagsman, 2004). The proteasomal degradation occurs in the cytosol and is inhibited in anaerobic metabolism (Voet and Voet, 2005). Proteolysis starts with the assembly of a monomeric protein, ubiquitin, with the ubiquitin-conjugating enzyme, a reaction catalyzed by the ubiquitin-activating enzyme. During this reaction, the ubiquitin-ligase enzyme binds with the protein to be degraded. Many ubiquitin—ubiquitin-conjugating enzyme complexes bind to a protein—ubiquitin-ligase enzyme with an isopeptidic bond, producing a polyubiquitin chain on the protein to be degraded (Los and Haagsman, 2004). Lastly, this complex is de-ubiquitinylated by the proteasome 26S, a multiproteic complex during degradation of the protein (Voet and Voet, 2005). Degradation by the proteasome machinery is relatively complete, with the protein being degraded into free amino acids and short polypeptides.

Apoptosis, programmed cell death, is triggered by caspases, which act through three different pathways, the intrinsic, extrinsic, and endoplasmic reticulum-mediated pathways, as described by Ouali et al. (2006). The caspases are from the family of

cysteine aspartate-specific proteases and 14 types have been identified (Earnshaw et al, 1999). They can be divided into three classes based on their biological functions: inflammatory, apoptosis initiation, and effector (Fuentes-Prior and Salvesen, 2004). Caspases have 280 substrates and are involved in inactivation and degradation of various proteins such as the myofibrillar and cytoskeletal protein (Fischer et al, 2003).

1.4.5 External factors affecting muscle development

A wide variety of factors influences muscle development and most of these factors act on the muscle fibre number and size. The environment, postnatal nutrition, species, selection pressure and breed type including genetic variability and heritability, gender, and physical activity are all known to affect muscle development and growth (Rehfeldt et al., 2004). These factors affect muscle development in different ways and to understand how growth affects meat quality, the specific effects of these factors need to be reviewed.

1.4.5.1 Gender

The relationship between gender and muscle fibre size is debatable. According to Lawrence and Fowler (2002), males usually have larger muscle fibres than females and castrated males. This relationship is based on the observed effect of sex on the rodent m. *rectus femoris* and m. *levator ani*, the bovine m. *longissimus*, and the chicken m. *extensor hallucis longus*. On the other hand, this sex-relationship has not been demonstrated with the canine m. *pectineus*, the porcine m. *longissimus*, and various muscles from rats and mice (Rehfeldt et al., 2004). Nevertheless, the sex-related myofibre difference is mostly explained by testosterone, a male sexual hormone, which has an anabolic effect on muscle fibres (MacIntosh et al., 2006) and stimulates satellite cell proliferation and muscle protein synthesis (Rehfeldt et al., 2004).

1.4.5.2 Postnatal nutrition

Muscle development cannot be normal with poor or under nutrition and usually results in reduced body and muscle weights. Postnatal muscle growth requires minimum levels of nutrients, otherwise the animal is considered to be undernourished. With

malnourishment, the amount of muscle proteins, muscle fibre diameter, muscle nuclei number (Rehfeldt et al., 2004), and DNA units (Pearson and Young, 1989) decrease.

Carcass traits of steers will differ depending on the feeding regimens with grain finishing and forage finishing yielding different carcasses. A high-energy diet fed to steers has been shown to increase their growth rate and slaughter weight compared to steers fed a medium-energy diet (Sinclair et al., 2001). Carcass traits and meat properties were both found to be affected by diet type by Kerth et al. (2007), with grain diet fed steers yielding the largest and fattest carcasses with unchanged or decreased SF in the ribeye and strip loin muscles respectively.

1.4.5.3 Selection pressure and breeds

Thousands of years ago, humans selected the animals they wanted to reproduce on the basis of how tame they were and how valuable they were as a source of food. These animals had specific characteristics that others within their species did not have. This intraspecies variation of traits is defined as genetic variability. With time, the populations controlled by humans have differed from the wild ones, becoming more similar within the controlled than in the wild population. The capacity to transfer characteristics from an individual to its progeny is called heritability (Rehfeldt et al., 2004). Hence, if animals are selected for their large musculatures, they will be more likely to have progeny with large musculatures than animals that were not selected for this characteristic.

Charolais, Hereford, Angus, and Red Angus are all from the species *Bos Taurus*, which differs from *Bos indicus* because they are humpless cattle that are not heat-tolerant (Buchanan and Dolezal, 1999). These breeds are classified into two groups: British, which originate from the British Isles, and Continental, which are from the European continent. The French Continental breed Charolais is a large framed animal that produces carcasses with high lean:fat ratios and is rated moderate to late for reaching puberty (Buchanan and Dolezal, 1999). The Hereford is a British breed with moderate size and age at puberty. Its low to moderate lean:fat ratio ranks this breed with more carcass fat than the Charolais breed, and its m. *longissimus thoracis* muscle has a mean marbling score of “small”, which is greater than the mean marbling score of “slight” associated with Charolais carcasses (Gregory et al., 1999). The moderately sized Angus and Red

Angus cattle are both from Scotland but Angus is specific to Aberdeen and Angus counties whereas the Red Angus is from Scotland in general (Buchanan and Dolezal, 1999). Angus cattle are rated moderate and low to moderate for age at puberty and lean:fat ratio, while Red Angus cattle are rated early to moderate and moderate for both of these traits, respectively. Angus cattle had the highest marbling score and were ranked second for carcass fat among nine Continental and British breeds (Gregory et al., 1999); however, it had the second lowest WBSF. In the same study, WBSF was slightly higher in Continental than in British breeds.

1.4.5.4 Environment

Photoperiod and environmental temperature are two main factors affecting animal muscle growth rate. Photoperiod is defined as the daily hours of light and dark to which animals are exposed. When photoperiod lengthens, food ingestion and growth rate increase. This phenomenon is an interaction between the endocrine system and hormone secretion and concentration (Hossner, 2005). With regard to environmental temperature, when animals are not in their thermal comfort zone, they must regulate their body temperature; hence, their energy is not devoted to protein synthesis, but rather to body temperature regulation. The environmental temperature also has an impact on food intake; cold stress improves food ingestion, whereas heat stress reduces food consumption. Energy ingestion and muscle and body weights are negatively affected in both cases (Rehfeldt et al., 2004). Therefore, the different management systems used in beef cattle production will affect cattle because seasons influence body regulation. Indeed, spring and fall born calves have been shown to have different growth patterns and food consumption habits by Kartchner et al. (1979). Marlowe and Gaines (1958) had the same observation and added that creep-feeding calves reduced the growth difference. Creep-feeding is to provide a calf with supplemental feed before weaning in addition to the dam's milk and pasture to increase the calf weight (Hand, 1998).

1.4.6 Internal factors affecting muscle development

Hormones and growth factors regulate muscle growth by endocrine, paracrine, autocrine, and neural transmission systems. Substances acting by endocrine transmission travel in the blood stream while the paracrine pathway uses the intercellular space for its

transmission. Neural transmissions use the nervous system and are closely linked to the endocrine transmission system (Lawrence and Fowler, 2002). Cells using the autocrine pathway produce substances that will act on themselves. Nonetheless, even though hormones and growth factors are present in the body, they need receptors to transmit their effects. This section presents a list of the main hormones implicated in growth that may mediate the effect of exogenous growth promotants.

1.4.6.1 Growth hormone

Growth hormone (GH), also known as somatotropin, has an anabolic effect on protein metabolism. GH is a single chain protein consisting of 191 amino acids with two disulfide bonds that maintain its three-dimensional structure. GH is synthesized by the pituitary gland, in the somatotrope cells; however, its secretion is controlled by the hypothalamus. In one day, approximately six to eight GH concentration spikes occur irregularly, although this can vary depending on species, sex, and age of animals. GH uses the endocrine pathway for its transmission (Hossner, 2005).

The release of GH has an indirect effect on muscle through the action of insulin-like growth factor-I (IGF) and it acts mainly via this growth factor in the body (Dauncey et al., 2004). When GH is released, it triggers the release of IGF-I from the liver. IGF-I then provides negative feedback on the hypothalamus, which reduces GH production. The main organs on which GH acts are the liver, adipose tissues, skeletal muscles, bones, and cartilage. GH improves muscle growth and glucose uptake by enhancing amino acid uptake and utilization, decreasing fat deposition, regulating carbohydrate metabolism (Hossner, 2005), and stimulating muscle fibre hypertrophy through activation of satellite cells and protein synthesis (Rehfeldt et al., 2004). Exogenous GH administered to cattle should maintain weight gain, increase protein gain, and reduce carcass fat (Ouali et al., 1987). Indeed, Dalke et al. (1992) observed reduced muscle fat content and increased protein and water contents in steers treated with recombinant bovine somatotropin implants. Also, serum concentrations of IGF-I increased after 28 days of receiving an implant every week.

1.4.6.2 Insulin-like growth factors

In skeletal muscles, insulin-like growth factors (IGF), or somatomedins, are mainly synthesized by the liver; however, they can also be synthesized by myoblasts, satellite cells, myofibres, and fibroblasts. IGF-I is a single-chain polypeptide formed by 70 amino acids of which tryptophan and histidine are absent. IGF-II is also a single-chain polypeptide, but it differs more frequently within species than IGF-I (Lawrence and Fowler, 2002). Various pathways are used for the transmission of IGF: endocrine, paracrine, and autocrine. IGF can be considered a system consisting of IGF-I, IGF-II, IGF receptors, and IGF binding protein (IGFBP) (Hossner, 2005). IGFBP-3 is the most important binding protein because IGF are mostly associated with it when circulating in the body. GH regulates the synthesis of IGF-I and IGFBP-3 (Dauncey et al., 2004), but IGF-I inhibits its own production by negative feedback on the hypothalamus (Hossner, 2005). IGF stimulates hypertrophy by instigating amino acid and glucose uptakes, inhibiting protein degradation, and improving protein synthesis (Hossner, 2005). IGF also induces slight proliferation of satellite cells (Allen and Boxhorn, 1989).

1.4.6.3 Sex hormones

Estrogens, progestins, and androgens are the primary hormones of reproduction and also serve in the development of secondary sexual characteristics. They belong to the steroid hormone family and use the endocrine pathway for their transmission. These hormones are synthesized by ovaries and testes and have cholesterol as a precursor (Bearden and Fuquay, 1997).

Androgens are synthesized by the Leydig cells in the testes and are divided into three hormones: testosterone, the main male sex hormone, androstenedione, and dihydrotestosterone (Bearden and Fuquay, 1997). Testosterone and its derivatives have an anabolic effect on skeletal muscle, which gives rise to their categorization as anabolic steroids (Hadley, 1996). Men treated with testosterone have shown increased muscle size (Bhasin et al., 1997) due to increased cross-sectional areas of type I and II fibres, muscle nuclei number (Sinha-Hikim et al., 2002) and satellite cell number (Sinha-Hikim et al., 2003). Testosterone also inhibits adipogenic differentiation (Singh et al., 2006); therefore, the lack of testosterone in castrated males is the reason why their carcasses contain more

fat and less lean tissue in comparison to those of intact males (Aberle et al., 2001). Muscles of the forequarter have a better response to androgens than muscle of the hindquarters as observable in muscles of the neck and crest region in uncastrated cattle, suggesting that the effect of hormones is mediated by receptors in the muscle cells rather than simply hormone concentration.

Androstenedione and testosterone are the precursor of estrogens (Hadley, 1996). This group is represented by three hormones, estradiol-17β, estriol, and estrone, with the first being the main female sex hormone (Bearden and Fuquay, 1997). Estrogens are produced by the granulosa cells of ovaries and testis, which can convert testosterone to estradiol. Progestins are secreted by the corpus luteum and include 20β-dihydroprogesterone, 17-hydoxyprogesterone, and progesterone. Excluding sexual effects, estradiol has an anabolic effect on the whole body, and thus causes weight gain, induces retention of H_2O and Na^+, and plays a role in the distribution of fat, whereas progesterone increases basal metabolic rate (Hadley, 1996).

1.4.6.4 Catecholamines

The catecholamines, epinephrine, norepinephrine, and dopamine, are hormones and neurotransmitters of the autonomic nervous system. This group of hormones acts by binding to adrenergic receptors embedded in the cell sarcolemma, of which there are two types, α and β, which are in turn subdivided into the classes α1-, α2-, β1-, β2-, and β3- (Hadley, 1996; Mersmann, 1998). α-Adrenergic receptors (α-AR) are present on vascular smooth muscles and they have a general contraction effect on all smooth muscles except those of the intestine. Activation of α-AR also stimulates fat storage and vasoconstriction of arteries, and inhibits insulin secretion. β-Adrenergic receptors (β-AR) are found on fat, kidney, lung, liver (Mersmann, 1998), skeletal muscles, cardiac, and brachial smooth muscles (Hadley, 1996). When stimulated, they induce a general relaxing effect on muscles with the exception that they stimulate cardiac muscle contraction (Hadley, 1996). Moreover, β-AR stimulate glucagon secretion and lipolysis, decrease proteolysis, and more specifically, β2-AR are bronchodilator and vasodepressors. When β-AR are stimulated, they link to adenylate cyclase and form cyclic adenosine monophosphate (cAMP), a messenger that transmits the action of β-AR in cells. Once the intracellular level of cAMP rises due to β-AR stimulation, desensitization of β-AR occurs within a few

seconds of stimulation, and no matter if the source of activation of β-AR is continuous, cAMP levels will stop increasing and plateau, and may decrease as well (Hadley, 1996).

1.4.7 Growth pattern modified through exogenous substances

Growth promotants are used in beef production to improve growth and weight gain of cattle. Although there are more than two methods of promoting growth of cattle, the most common methods are the administration of hormonal growth implants and the feeding of β-adrenergic agonist substances.

1.4.7.1 Hormonal growth implants

Synthetic hormones are used in beef production to improve the rate and efficiency of weight gain. Hormones are administered in the form of implants injected under the skin of the ear. Hormonal implants in Canada contain different concentrations of natural hormones such as progesterone, testosterone, estradiol-17ß, and/or synthetic hormones like trenbolone acetate, zeranol, and melangestrol acetate (CFIA, 2010). Other than the mechanisms presented in Section 1.4.6.3, the mode by which these hormones act is not fully understood but they are thought to stimulate the secretion of GH (Aberle et al., 2001), which in turn acts through IGF-1 and stimulates satellite cell proliferation and protein synthesis in skeletal muscles (Rehfeldt et al., 2004). Administration of trenbolone acetate and estradiol implants is capable of increasing serum concentrations of IGF-I (Johnson et al., 1996; Walker et al., 2007), IGFBP-3 (Johnson et al., 1996) and IGF-I mRNA abundance (Pampusch et al., 2003). Nevertheless, findings of Pampusch et al. (2008) suggested that only estradiol was responsible for the increase in IGF-I mRNA level and not trenbolone acetate because both estradiol and estradiol/trenbolone acetate implants increased the circulating IGF-I mRNA level, but not trenbolone acetate administered alone.

1.4.7.2 β-Adrenergic agonists

β-Adrenergic agonists (β-AA) are synthetic phenethanolamine derivatives (Moody et al., 2000) that have similar effects to catecholamines (Hossner, 2005). β-AA are fed orally to cattle and take action by binding to the β-AR of various tissues in a manner

similar to that of the catecholamines (Hossner, 2005). β-AA decrease fat deposition, improve feed efficiency, and increase rate of weight gain, lean growth, and protein deposition. The augmentation of protein deposition is caused by a reduction in protein degradation rather than an intensified protein synthesis in the protein synthesis-degradation balance (Hossner, 2005). Also, carcasses from cattle fed a β-AA are leaner than those from cattle which have not received a β-AA as it induces hydrolysis of triglycerides (Chang, 2007).

β-AA do not act through IGF-I in cattle like sexual hormone implants because IGF-I circulating concentration and mRNA abundance have been shown not to be affected by the β-AA ractopamine hydrochloride (RAC) (Winterholler et al., 2008) or to decrease or to tend to decrease respectively following RAC feeding (Walker et al., 2007). Also, β-AR and GH receptors do not have similar structures; therefore, β-AA would not be able to activate the GH receptors (Mersmann, 1998).

β-AA also appear to shift muscle fibre type because a shift from type I to type IIX fibres was demonstrated in the m. *soleus* of rats administered clenbuterol, and from type I to type IIB fibres when fenoterol was administered (Ryall et al., 2002), although the mechanism involved in fibre shift remains unsolved (Chang, 2007). This phenomenon might be related to the abundance of β-AR on muscle fibres, because slow fibres have a greater density of β2-AR than fast fibres (Williams et al., 1984), and the β-AA selectivity for β-AR. Cimaterol, clenbuterol, salbutamol, zilpaterol, and $L_{644,969}$ are β2-AR specific compounds while RAC is β1-AR specific (Moody et al., 2000). Nevertheless, some studies suggest that RAC can alter the expression of β2-AR in cattle (Sissom et al., 2007; Winterholler et al., 2007).

1.5 POST MORTEM CHANGES IN MUSCLES

Post mortem changes occurring in muscles are both physical and chemical. These changes are partly caused by rigor mortis, a phenomenon by which muscles become rigid and inextensible, and post mortem ageing, during which time muscles will further transform into meat. Post mortem changes due to rigor mortis and ageing were well described by Wheeler and Koohmaraie (1994), who found that cooked meat toughness

increased during the first 24 hours post-mortem in the lamb m. *longissimus thoracic et lumborum* but decreased markedly in the following 24 hours with refrigerated ageing.

1.5.1 Chemical changes and rigor mortis

Chemical changes in muscles are principally dependent on the supply of energy to muscles. The diverse reactions occurring after death are like a chain reaction. First, the metabolism of muscles cannot remain the same because the blood stream stops, which also stops the supply of oxygen to muscles. Anaerobia is created and the oxidative metabolism of muscle ceases, leaving only the glycolytic metabolism active in the cytosol (Pearson and Young, 1989). In an aerobic muscle environment, the oxidative metabolism is active and the mitochondria use nicotinamide adenine dinucleotide, pyruvic acid, lactic acid, fats, and amino acids to produce ATP by β-oxidation, the citric acid cycle, the electron transfer chain, and oxidative phosphorylation (MacIntosh et al., 2006). Nevertheless, the electron transfer chain that requires oxygen to function and produce ATP is inactivated post mortem, ceasing the supply of ATP to muscles by mitochondria. ATP can only be produced by anaerobic glycolysis. It is further used to maintain fluidity between actin and myosin, and to maintain cytosolic calcium concentration through the Ca^{2+}-ATPase, functions that continue post mortem for as long as ATP is supplied.

Glycolysis during anaerobia uses two ATP and one glucose molecule from glycogen as substrates to produce four ATP in an attempt to maintain the other vital functions of muscles and muscle homeostasis. Anaerobic glycolysis produces two lactic acid molecules, two H^+ protons, and two ATP for every molecule of glucose. Lactic acid and H^+ protons accumulate in the muscle sarcoplasm because the blood supply that normally facilitated their removal to the liver is no longer available (Aberle et al., 2001; Gerrard et al., 1987). ATP is also produced from the reaction between adenosine diphosphate (ADP) and phosphocreatine, a storage molecule of high-energy phosphate, which is catalyzed by the creatine kinase enzyme (MacIntosh et al., 2006). Glycolysis can still provide some ATP when the content of phosphocreatine drops, but inefficiently (Lawrie and Ledward, 2006). Once the ATP level declines, rigor mortis begins. This fast phase of rigor is marked by a pH decline due to the accumulation of H^+ protons produced from glycolysis, which acidifies muscles to ultimate pH values of about 5.5 (Lawrie and Ledward, 2006). Ruiz de Huidobro et al. (2003) observed that pH values dropped rapidly

in the bovine m. *longissimus thoracis et lumborum* during the first hours of storage, from 6.5 to 5.5 in 24 hours.

Actin and myosin also use ATP post mortem to maintain a relaxed and fluid state of readiness, contributing to the depletion of ATP early after death. Random nerve firings post mortem prompt thin and thick filaments to contract and this sliding contraction requires energy, which is provided by the ATP hydrolysis performed by the myosin heads (Lehninger, 1982). When ATP is hydrolyzed, actin and myosin return to an unlocked form (MacIntosh et al., 2006). Otherwise, with a lack of ATP, the actomyosin complex is formed and rigor mortis begins (Pearson and Young, 1989). The actomyosin complex does not allow movement of muscle; consequently the muscle appears to lose its extensibility (Lawrie and Ledward, 2006).

Another post mortem consumer of ATP is the Ca^{2+}-ATPase. This is an enzyme located in membranes of the sarcoplasmic reticulum that hydrolyses ATP to pump calcium from the muscle sarcoplasm into the sarcoplasmic reticulum. The role of the Ca^{2+}-ATPase is to keep the concentration of Ca^{2+} at less than 10^{-8} M in the cytosol to preserve cell functions even in the post mortem phase (Pearson and Young, 1989).

1.5.2 Factors affecting post mortem glycolysis

Rigor mortis is completed once ATP is exhausted. Results from Etherington et al. (1987) showed that rigor mortis was completed at 15°C after four hours for chicken carcasses, ten hours for pig carcasses, 15 hours for lamb carcasses, 18 hours for calf carcasses, and 29 hours for beef carcasses. The length of time before rigor mortis is completed is important in the determination of the meat quality; therefore, factors that affect glycolysis rate also affect the time to rigor mortis completion.

The temperature at which muscle is chilled early post mortem affects glycolysis through its effect on glycolytic enzyme activities. High post mortem muscle temperatures increase glycolytic enzyme activities in muscles, instigating an accelerated muscle pH drop. With low post mortem muscle temperatures, glycolytic enzyme activity is reduced so glycolysis is delayed and the muscle pH decline is slowed (Pearson and Young, 1989). For example, at 37°C, rigor mortis can last from less than half hour with exhausted

animals to four hours with well-fed animals, whereas one to ten hours may be required for those types of animals when the carcasses are stored at 20°C (Bendall, 1973).

Variations in glycolytic rate can also exist between muscles types. For example, the beef m. *longissimus dorsi* is more active than the beef m. *triceps brachii* during the death struggle (Bendall, 1978), thus the m. *longissimus dorsi* has a higher glycolytic rate and a faster pH decline than the m. *triceps brachii*. Fast glycolysing muscles generate more heat than slow glycolysing muscles because of their increased glycolysis rate (Ringkob et al., 1989). This was observed by Smulders et al. (1990) who found that at three hours post mortem m. *longissimus* having a pH higher than 6.3, which qualified as slow glycolytic muscles, had lower internal temperatures than fast glycolysing m. *longissimus,* which had pH values less than 6.3.

Pre-slaughter stresses affect muscle contents of high-energy phosphate, ATP, ADP, and phosphocreatine. As observable in beef, the exhaustion of cattle before slaughter ends in dark, firm, and dry (DFD) meat because the muscle glycogen content is depleted and remains at a low level at the time of slaughter (Pearson and Young, 1989). When glycogen content is low in stressed cattle, little glycolysis occurs after death and so less lactate and fewer protons are produced than usual. Thus, the decline of the muscle pH is not as marked as it should be and muscle pH remains at values at or above 6.0. The problem is that without a normal pH decrease, the meat has reduced flavour and is prone to micro-organism spoilage, which compromises preservation and shelf-life, although it tends to be more tender than meat with a normal pH (pH 5.5 to 5.7) (Silva et al., 1999). DFD meats, along with pale, soft, and exudative (PSE) meats are syndromes altering meat quality, thereby affecting the meat industry (Pearson and Young, 1989) although they are much less prevalent than in past decades. PSE meat results from a tremendously rapid anaerobic glycolysis post mortem (Pearson and Young, 1989) and is mostly observable in pork. The high muscle temperature combined with the low pH of rapid glycolysis cause denaturation and precipitation of sarcoplasmic protein onto the myofibrillar proteins (Bowker et al,. 2000).

Physical changes also occur in meat post mortem and are more easily observable and measurable than chemical changes during rigor mortis because they affect the appearance and sensory properties of the meat. These changes are considered to be indicators of meat quality in research and are quantified using measurements such as shear force (SF), objective colour measurement, myoglobin relative contents, water-holding capacity, and sarcomere length. SF is commonly evaluated with a Warner-Bratzler mechanical device in research because it is less expensive than sensory panels. SF is dependent on many physical aspects in meat; therefore it will be discussed throughout the following sections.

1.6.1 Degradation of tissues

Studies with cattle carcasses that have used different lengths of time for post mortem tenderization suggested that most of the meat tenderization occurred during the first six days post mortem (Calkins and Seideman, 1988), the first week (Monsón et al., 2004), or the first 14 days (Monsón et al., 2005). Gruber et al. (2006) found that discernible post mortem changes in muscle toughness can continue to occur for 21 and 28 days. The proteolytic alteration of myofibrils involves proteases of which cathepsins, calpains, and proteasomes may be involved. Connective tissue is also degraded post mortem but not by the same proteases. As discussed earlier, the matrix metalloproteinases are most likely involved in its degradation. The degradation of tissues is closely related to meat tenderness, so proteolytic enzymes will be reviewed within this context even though degradation of tissues is not the only factor affecting meat tenderness.

1.6.1.1 Calpains

Both the m- and μ-calpains are thought to participate in post mortem proteolysis (Hopkins and Taylor, 2004), especially in the first 72 to 96 hours post-mortem (Taylor et al., 1995). The failure of the Ca^{2+} ion pumps after death activates the calpains, or calcium-active proteases, by the equilibration of the concentration in Ca^{2+} free ion throughout muscle tissues (Etherington, 1984). Etherington et al. (1987) determined that

µ-calpain accounted for 64% of the calpain activities in muscles, thus the ration of µ:m calpains would be around 1.8:1. The optimal pH range for the activity of calpains is 6.5 to 8.0 (Etherington, 1984). Beef steaks from m. *longissimus* of ultimate pH values ranging between 6.1 and 6.5 at one day post mortem were more tender than meat of ultimate pH values of 5.7, a difference explained by the proteolytic enzymes being more active at pH values of 6.1 to 6.5 than at 5.7 (Silva et al., 1999). m-Calpain activity can persist for up to three days post mortem without being altered; therefore, its activity remains relatively consistent during rigor (Ducastaing et al., 1985). Activity of the µ-calpain, which is the calpain thought to be mainly involved in the tenderization of meat (Koohmaraie, 1996), diminishes by about 50% during rigor mortis (Ducastaing et al., 1985). The calpains have been shown to degrade post mortem actin, myosin light chain, troponin T (Houbak et al., 2008), titin, desmin, troponin I (Koohmaraie et al., 2002), vinculin, nebulin (Taylor et al., 1995), synemin (Bilak et al., 1998), and vimentin (Nelson and Traub, 1983).

The level of calpastatins, the inhibitors of calpains, has also been positively correlated with SF in bull meat at five and twelve days post mortem by Steen et al. (1997). Koohmaraie et al. (1991) showed that the calpastatin:µ-calpain ratio is around 4:1 in beef m. *longissimus*. Geesink and Koohmaraie (1999) showed that even with a calpastatin:µ-calpain ratio higher than 4:1, proteolysis by calpains was still occurring, although µ-calpain activity also depended on the ionic strength-dependent instability of autolyzed µ-calpain, temperature, pH, and the presence of Ca^{2+}. The extent of calpain inhibition by calpastatin post mortem is more important in *Bos Indicus* than in *Bos Taurus* breeds and crossbreds. A study conducted by Whipple et al. (1990) showed that cooked meat from Angus-Hereford crossbred steers had a lower mean WBSF at 24 hours post mortem than that from Sawihal-Angus-Hereford crossbred steers. The high level of calpastatin 24 hours post mortem in these latter steers was responsible for the great toughness of their meat.

1.6.1.2 Cathepsins

The cathepsins B and L are thought to be the cathepsins most likely to be active in post mortem proteolysis (Ouali et al., 1987). During rigor mortis, the cathepsins may be released from lysosomes in the cytosol due to the sarcoplasmic pH decline and the failure

of the ion pumps in the sarcoplasmic reticulum membranes (Pearson and Young, 1989). Ultimate pH values of muscles vary between 5.3 and 5.8 (Bendall, 1973) which is suitable for cathepsin activities because the pH range that suits types B and L activities is pH 3.0 to 6.0 (Etherington, 1984). Myosin, actin, α-actinin, the troponins, and tropomyosin are among the proteins that can be degraded by the cathepsins (Obinata et al., 1981).

If the cathepsins are involved in the meat tenderization process, their activity may be curtailed by cystatin, which is the protein that binds to and inhibits cathepsins. Indeed, immediately after the release of the cathepsins from the lysosomes, cystatin forms a complex with the cathepsins making these unavailable (Etherington et al., 1987). These authors also observed that the cathepsin inhibitors blocked as much as 70 to 75% of the cathepsins released from lysosomes, and that cathepsin L was blocked more than cathepsin B. Johnson et al. (1990) found cathepsin B and L activities were negative correlated with WBSF the first ten days post mortem while results of Calkins and Seideman (1988) showed a correlation between the activities of the cathepsins B and H and the reduction of SF from day three to six. Hence, from these studies, we can infer that cathepsins may be active proteolytically throughout ageing, but meat tenderization resulting from this proteolysis may be perceived only after a few days of ageing.

1.6.1.3 Proteasomes

The proteasomes were long thought to not participate in post mortem tenderization (Koohmaraie, 1992) partly because this complex is inactivated in anaerobic metabolism (Voet and Voet, 2005). Recent studies of the 20S proteasome however reported that it was involved with the tenderization of meat (Dutaud et al., 2006; Houbak et al., 2008; Lamare et al., 2002). Lamare et al. (2002) suggested that tenderization cannot be accomplished by the calpains and the cathepsins alone due to calcium concentration restrictions and time limitation of their activity. This implied that the proteasomes were synergistic to the other proteolytic systems because proteolytic degradation was sequential and the proteasomes required the initiation of sarcomere disruption by the calpains before they could be effective (Houbak et al., 2008). The 20S proteasome is located on the I-band and preferentially degrades proteins located in the Z-discs and I-

bands (Dutaud et al., 2006), specifically actin, nebulin, and troponin T (Houbak et al., 2008).

1.6.1.4 Caspases

Implication of caspases in meat tenderization as been suggested by some authors; however, the relationship between meat tenderness and caspases is not well described in the literature. Hypoxia that occurs post mortem is thought to release caspases and cause apoptosis of muscle cells (Gustafsson and Gottlieb, 2003). These proteases could therefore be active in early post mortem tenderization of meat (Sentandreu et al., 2002). A study by Kemp et al. (2006) showed that the caspase activities were highest two hours post mortem and decreased significantly after 32 hours post mortem. Moreover, depending on the caspase type, correlation between SF and caspases were significant (caspase 9) or tended to be significant (caspases 3/7) and were negative. Caspase 3 has however been shown by Underwood and Means (2008) to not be involved in postmortem meat tenderization based on its activation and lack of correlation with WBSF.

1.6.1.5 Matrix metalloproteinases

Connective tissue is one of the main factors proposed to contribute to beef toughness (Bouton et al., 1975; Koohmaraie et al., 2002; Møller, 1980). Matrix metalloproteinases (MMP), initially described in Section 1.3.5, are involved in collagen turnover during life through degradation of the connective tissues (Purslow, 2005). Sylvestre et al. (2002) observed that lamb collagen degradation may be performed by MMP-2 because its level was correlated with collagen insolubility both at slaughter and at 21 days post mortem. In another study, Pambuka et al. (2007) isolated a MMP from ostrich skeletal muscles and found its specific activity increased after 21 days post-mortem, but no change was observable during the first nine days. No weakening of the intramuscular connective tissue before ten days of ageing was noted by Nishimura et al. (1998) in muscles from beef steers, but further weakening was observable until 35 days post mortem. These authors suggested that during the first ten days of ageing meat tenderization was due to the weakening of myofibrillar structures, and after ten days to the weakening of the endomysium and perimysium. Therefore, post mortem weakening

of the intramuscular connective tissue by MMP may be expected to be perceived after a certain lapse of time only.

1.6.2 Sarcomere length and shortening

Sarcomere shortening occurs during rigor mortis with the formation of the actomyosin complex, which results from the overlapping of actin and myosin (Lawrie and Ledward, 2006). Takahashi et al. (1995) showed that sarcomeres shortened and reached a minimum size around two to three days post mortem but lengthened afterward, although not to the same length as at one hour post mortem.

Sarcomere lengths are quite variable and depend upon many factors such as the post mortem glycolytic rate, early post mortem muscle temperature, and the rate of early post mortem muscle pH decline. For example, ageing of meat at 35°C reduced sarcomere length compared to ageing at 18°C (Devine et al., 2002). Moreover, sarcomere lengths appear related to pH values as well because different ultimate pH values result in different magnitudes of shortening. A study on the m. *longissimus dorsi* of steers and bulls showed that SF increased as ultimate pH increased and the author explained that the sarcomeres shortened from 1.64 to 1.51 µm with an increase in ultimate pH from 5.5 to 6.2 (Purchas, 1990). As for the glycolytic rate, muscles classified as slow glycolytic muscles reached their ultimate pH slower and had shorter sarcomeres two days post mortem than intermediate or fast glycolysing muscles (O'Halloran et al., 1997).

Nevertheless, meat toughening is only partially due to sarcomere shortening. Herring et al. (1965), Koohmaraie et al. (1996), Seideman et al. (1987), and Steen et al. (1997) all observed increased SF with shortening of sarcomeres; however, Seideman et al. (1987) observed that the sarcomere length was not correlated with tenderness until sarcomeres were shorter than 1.8 µm. On the other hand, decreased sarcomere length was not always associated with changes in tenderness (Fiems et al., 1990; Hall and Hunt, 1982; Johnson et al., 1990). So, the influence of sarcomeres on meat toughness depends on the extent of the shortening although other post mortem factors such as proteolysis are probably more determinative of tenderness than sarcomeres.

1.6.3 Colour and myoglobin

Colour is the most important factor affecting consumer meat buying decisions (Boles and Pegg, 2005; Millar et al., 1994). Therefore, meat from cattle produced using different management systems or that received various exogenous growth promotants should still fulfill the expectations of consumers in terms of colour.

One of the colorimetric systems widely used to measure meat colour is the three-dimensional CIELab system. When measuring colour using this scale, three basic parameters are measured: lightness (L*) (0=black, 100=white), a* (redness/greenness axis) with positive values being indicators of red and negative values indicating green, and b* (yellowness/blueness axis) with yellow represented by positives values and blue with negative values (Schanda, 2007). There are two other parameters in this system, hue and chroma, which are calculated from a* and b* with the following equation: hue = arctan (b* / a*), and chroma = $\sqrt{(a^{*2} + b^{*2})}$. Hue describes a colour being from red, yellow, green, blue, or a combination of two (Schanda, 2007), and chroma is the saturation or purity of a colour with high values associated with a high colour saturation (Aberle et al., 2001).

Changes in meat colour are attributable to changes in meat ultimate pH, protein structure, and myoglobin state. Practical observations of the relationship between these three parameters and meat colour are DFD and PSE meat. With DFD meat, the ultimate pH is higher than 6.0 and close to that of normal physiological pH (pH 6.9 to 7.4) so that proteins remain close to their native state. The relatively intact myofibrillar proteins of DFD meat have a low light reflectance, which causes the meat to appear dark (Kinsman et al., 1994). The opposite is true for PSE meat which usually has a very low ultimate pH value (pH < 5.4), high light reflectance, and appears pale (Boles and Pegg, 2005). Abril et al. (2001) showed that, at 24 hours post mortem, as ultimate pH decreased, L* increased, meaning that the darkness of meat increased as ultimate pH value increased. Moreover, meat with an ultimate pH greater than or equal to 6.1 had lower L*, a*, b*, chroma, and hue values at zero and ten minutes, five hours, two, six, and nine days post mortem than meat with an ultimate pH lower than 6.1 except for a* at six and nine days (Abril et al., 2001). Lightness can also be indicative of protein denaturation because as proteins denature, water-holding capacity decreases and moisture losses (drip loss) increases

(Penny, 1977), and as drip loss increases, L* increases (Ryu and Kim, 2006; Steen et al., 1997) and proteins denature.

The pigment myoglobin is also indicative of meat colour but this depends on the state of the pigment. When meat is freshly cut, deoxymyoglobin (DMB) gives the muscle a dark purple colour. Once the meat oxygenates or blooms, the myoglobin protein is oxygenated and forms oxymyoglobin (OMB), which produces a bright red colour (Boles and Pegg, 2005). Millar et al. (1994) evaluated the formation of OMB in beef meat and at 24 hours of exposure to oxygen, OMB was found at a tissue depth of between 3.57 to3.60 mm deep and between 10.17 and 11.00 mm deep after 48 hours. Iron on DMB and OMB, in a ferrous state (Fe^{2+}), can also be oxidised, meaning to lose an electron. Iron turns into a ferric state (Fe^{3+}) and form metmyoglobin (MMB) with the meat colour turning brownish when about 60% of the myoglobin reaches this state (Brooks, 1938). The myoglobin states can change from one to another but once the MMB state is reached, the reaction is difficult to reverse under natural conditions (Boles and Pegg, 2005). The formation of MMB is not desirable for consumers because its brownish colour is often incorrectly perceived to result from bacterial spoilage, but consumers base their choices on the appearance of meat (Smith et al., 2000). Therefore, the formation of OMB, associated with a red colour, is preferred and reflects a fresh and wholesome beef product (Troy and Kerry, 2010).

1.6.4 Water-holding capacity

In muscles, 85% of water is retained in the myofibrils and the remaining portion is located between the myofibrils, between the myofibres, and between the muscle bundles (Huff-Lonergan and Lonergan, 2005). As a polar molecule, water is free, immobilized, or bound to proteins in muscles with the latter form accounting for less than 10% of the water in muscles (Huff-Lonergan and Lonergan, 2005). Unlike bound water, immobilized water is not tightly bound to protein but rather held into muscle filamentous structures sterically or by attraction to bound water. As a consequence, immobilized water can move but not freely (Huff-Lonergan and Lonergan, 2005). Free water is only held by weak surface forces (Huff-Lonergan and Lonergan, 2005) and is most likely the source of purge or drip from sliced meat.

During rigor mortis, sarcomere shortening reduces the space available for water, expelling a fraction of the water from the sarcomeres (Bendall, 1973). Also, the decline in pH causes the muscle proteins to approach their isoelectric point, which reduces their electric charge and decreases their ability to bind water molecules, releasing free and immobilized water (Lawrie and Ledward, 2006). The isoelectric point of myosin, which is pH 5.4, is the point at which the quantity of water attracted diminishes and the internal structures of myofibrils are closely aligned, thus reducing the space between myofilaments and forcing water from between the myofibrils (Huff-Lonergan and Lonergan, 2005). After 24 to 48 hours post mortem, the release of water from the muscle is facilitated by drip channels that are formed by gaps between fibre bundles and the perimysial network (Offer and Cousins, 1992). Water losses are a financial concern for meat retailers because they reduce the saleable weight of meat products (Cheng and Sun, 2008).

The ability of muscles to retain water post mortem is called the water holding capacity and it is estimated by measuring how much immobilized water is released (Huff-Lonergan and Lonergan, 2005). Water dripping from raw meat is called drip or purge loss, whereas water lost during the cooking of meat is termed cooking loss. Drip or purge loss is mainly due to the rate of pH decline post mortem and the denaturation of sarcoplasmic proteins, but it is also affected by the ultimate pH, intramuscular fat content, species, age, conditioning of meat, and muscle type (Lawrie and Ledward, 2006). Drip losses result in weight loss, which is an economic concern (Offer and Cousins, 1992), but the drip can also contain protein and represents a protein loss as well. Indeed, sarcoplasmic protein loss can amount to as much as 12% of the absolute protein amount for certain proteins in pork meat (Savage et al., 1990).

Cooking losses are mainly water (Heymann et al., 1990) but also consist of soluble protein and fat (Cheng and Sun, 2008). The loss in water content of cooked meat is explained by the heat of cooking evaporating the water (Aberle et al., 2001), denaturing the proteins of meat (Cheng and Sun, 2008), shrinking the collagen fibres (Rao et al., 1989), and reducing the sarcomere length (Bouton et al., 1975). These structural changes result in the expulsion of water from the myofibres (Lawrie, 1998). Ultimate pH values were also associated with cooking losses in a study by Purchas et al. (1990), who found that as muscle pH decreased cooking losses increased. In low ultimate pH meat, ranging

between 3.9 and 4.5, connective tissue shrinkage during cooking was responsible for water losses (Rao et al., 1989). The protein denaturation during cooking starts between 50 and 60°C, the temperature range in which myofibrillar proteins, particularly myosin, denature (Findlay et al., 1986). Denaturation of collagen fibres starts at about 65°C (Bailey and Light, 1989) and is completed by about 70°C (Brunton et al., 2006). Actin is more heat resistant than myosin because it is denatured at about 81°C (Findlay et al., 1986). The formation of gelatin from collagen denaturation depends on the formation of cross-links and their nature. Heat-stable cross-links do not dissolve as easily as heat-labile aldimine cross-links associated with young animals, thus denaturation and gelation of collagen is less likely to happen in mature animals (Bailey and Light, 1989).

1.7 CONCLUSION

Growth relies upon many factors that are either endogenous or exogenous and this last category can easily be controlled by humans. The resultant effect will be reflected in the bodies of growing cattle by changes in size, proportion, and composition of muscles. Muscles are of prime importance in livestock production because they will become the final saleable product, which is meat. It is therefore to the advantage of producers to obtain the most meat they can from each individual animal with the lowest cost possible by optimizing production conditions. From the literature, a production system that favours increased tenderness of meat is reducing the age of the animal at slaughter, as the toughening effect of collagen should be reduced (Boccard et al., 1979). The use of hormonal growth implants, however, has been associated with decreased beef tenderness (Faucitano et al., 2008; Foutz et al., 1997) and this practice is prevalent in the Alberta beef industry.

With the recent approvals of ractopamine hydrochloride (RAC) and zilpaterol hydrochloride (ZH) by Health Canada and the Canadian Food Inspection Agency, β-AA may soon become as popular as hormonal implants. Nevertheless, RAC and ZH have to be managed differently because ZH has more aggressive effects on muscle tissue than RAC (Strydom et al., 2009), so data on ZH cannot be fully applied to predict the outcomes of using RAC. Hormonal implants and β-AA use different mechanisms in the body to stimulate growth but the results in both cases are an increase in protein accretion in muscles. Biological mechanisms are altered when growth is artificially promoted;

therefore, muscles can be affected in the sense that their biological, chemical, and physical properties can be modified. β-AA have also been implicated in the toughening of beef (Avendaño-Reyes et al., 2006; Claus et al., 2010; Gruber et al., 2008; Strydom et al., 2009), but the significant economic advantage they offer with great carcass yield may overshadow meat quality concerns. In order to use these production tools effectively, the interactions between hormonal implants, β-AA, and other production factors such as age and breed need to be examined.

The synergy of hormonal implants and β-AA has been studied by Bass et al. (2009) and the mean hot carcass weight of steers was significantly improved with RAC and three different hormonal implants, with a different magnitude of response across implant strategies. Contradicting these authors, yearling steer hot carcass weights in the study of Winterholler et al. (2008) were not improved by both RAC and a hormonal implant. Therefore, whether the hot carcass weight is improved or not by the use of both hormonal implants and RAC has to be elucidated and this can be partially addressed by studying individual muscles. Moreover, Bass et al. (2009), Winterholler et al. (2008), and other studies on RAC and hormonal implants (Gonzalez et al., 2007; Sissom et al., 2007; Walker et al., 2007) did not include a meat quality analysis in their study. So, it is unclear whether these growth promotants are synergistic or not with respect to growth, muscle properties, and meat quality.

The research program described in this thesis examined the effects of animal age at slaughter, implantation, RAC supplementation, and breed crosses on quality in two beef muscles with similar fibre types but differing in their connective tissue content. Furthermore, because meat tenderness is a concern for the meat science community, an analysis to further examine the yield points in the WBSF deformation curve was performed to investigate if connective tissue or myofibrillar proteins were the basis of any changes in beef tenderness observed. Indeed, to better understand cooked beef tenderness, we have to know what toughens meat, why, and how it happens in the muscle. From there, this may help us understand the factors involved when meat toughening occurs with different management systems.

1.8 TABLES

Table 1-1 Average composition of skeletal muscle

Component	Amount (% by wet weight)
Water	75.0
Proteins	19.0
Lipids	2.5
Carbohydrates	1.2
Soluble non-protein substances	2.3

(Pearson and Young, 1989)

Table 1-2 Collagen classification

Collagen families	Type of collagen	Molecular composition[1]	Location
Fibril-forming	I	$([\alpha1(I)]_2\alpha2(I))^2$	Bone[2], striated muscles[3], cornea[4], skin[5], tendons[5], blood vessels[5]
	II	$([\alpha1(II)]_3)^2$	Cartilage[5], vitreous humor[2]
	III	$([\alpha1(III)]_3)^2$	Striated muscles[3], skin[5], blood vessels[5]
	V	$([\alpha1(V)]_2\alpha2(V))$; $([\alpha1(V)]_3)^2$	Teeth[2], bone[2], placenta[2], skin[2], smooth[2] and striated muscle[3], cornea[4]
	XI	$(\alpha1(XI)\alpha2(XI)\alpha3(XI))^6$	Cartilage[7]
	XXIV	$(\alpha1(XXIV))^7$	Developing bones[7], cornea[7]
	XXVII	$(\alpha1(XXII))^7$	Cartilage[7], eyes[7], ears[7], lung[7]
Fibril-associated collagens with interrupted triple-helix (FACITs)	IX	$(\alpha1(IX)\alpha2(IX)\alpha3(IX))^2$	Cartilage[7], vitreous humor[2]
	XII	$([\alpha1(XII)]_3)^8$	Tendons[8], ligaments[8], skin[8], cornea[8]
	XIV	-	-
	XVI	-	No distinct distribution[8]
	XIX	-	Cell basement membranes[3]
	XX	$(\alpha1(XX))^7$	Corneal epithelium[7], tendons[7], skin[7], cartilage[7]
	XXI	$(\alpha1(XXI))^7$	Many tissues[7]
	XXII	$(\alpha1(XXII))^7$	Tissue junctions[7]
Short chain	VIII	$(\alpha1(VIII))^9$	Sclera[10], skin[10], striated muscles[3]
	X	$([\alpha1(X)]_3)^6$	Endochondral growth plate[11]
Beaded filament forming	VI	$(\alpha1(VI)\alpha2(VI)\alpha3(VI))^8$	Cell basement membranes[3],cornea[4]
Basement membrane and associated collagens	IV	$([\alpha1(IV)]_2\alpha2(IV))^2$	Basal lamina[2]
	VII	$([\alpha1(VII)]_3)^{12}$	Anchoring fibrils[9]
	XV	$(\alpha1(XV))^{12}$	Cell basement membranes[3]
	XVIII	$(\alpha1(XVIII))^{12}$	Cell basement membranes[3]

With transmembrane domains	XIII	$([\alpha1(XIII)]_3)^2$	Skin hemidesmosomes[2]
	XVII	$([\alpha1(XVII)]_3)^2$	Skin hemidesmosomes[2]
	XXIII	$(\alpha1(XXIII))^7$	Metastatic tumour cells[7]
	XXV	$(\alpha1(XXV))^7$	Neurons[7]
Other	XXVI	$(\alpha1(XXVI))^7$	Testis[7], ovaries[7]
	XXVIII	$(\alpha1(XXVIII))^{12}$	Sciatic nerve[12]

[1] Molecular composition refers to the three α-chains constituting a collagen molecule
[2] (Lodish et al., 2005)
[3] (Kovanen, 2002)
[4] (Kern et al., 1991)
[5] (Lapiere et al., 1977)
[6] (Bailey and Light, 1989)
[7] (Myllyharju and Kivirikko, 2004)
[8] (Hulmes, 2008)
[9] (Kühn, 1987)
[10] (Shuttleworth, 1997)
[11] (Rucklidge et al., 1996)
[12] (Veit et al., 2006)

Figure 1-1 Structure of a sarcomere

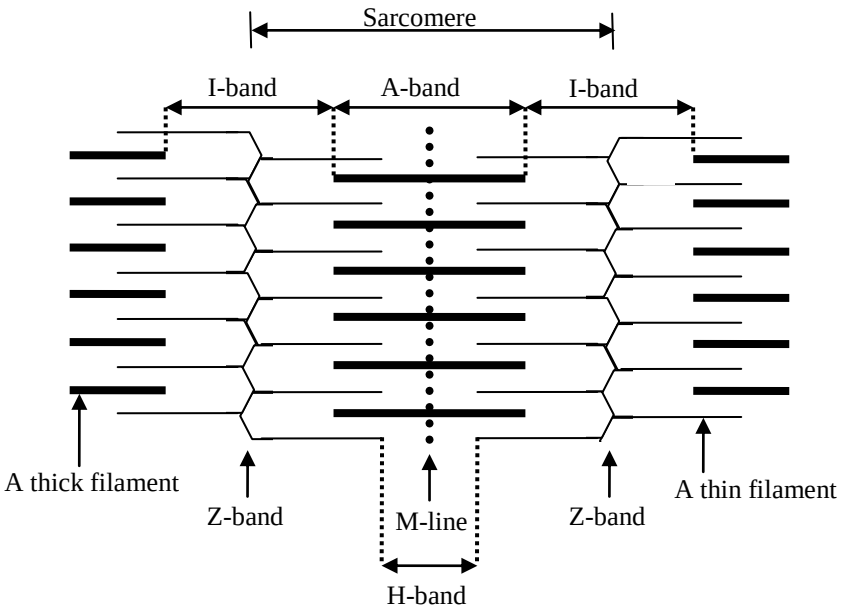


Figure 1-2 Lateral arrangement of procollagen molecules

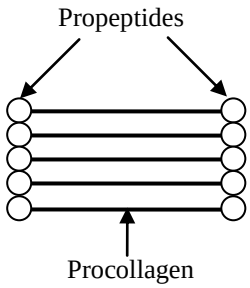


Figure 1-3 Staggered arrangement of collagen molecules forming a collagen fibril

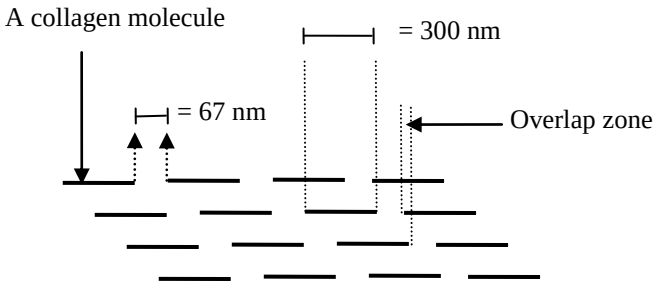
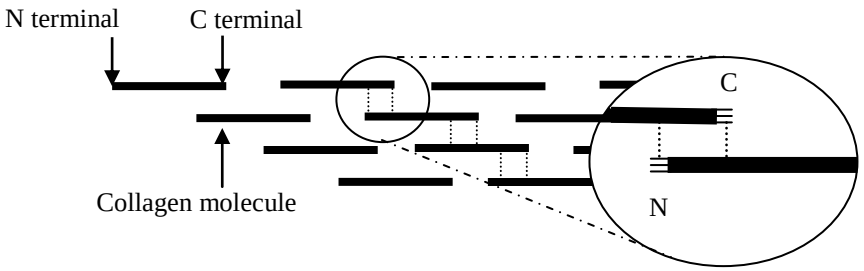
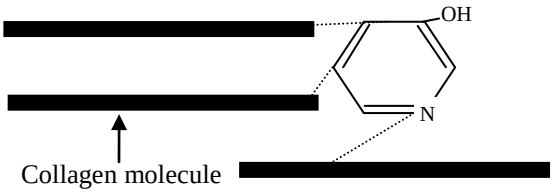


Figure 1-4 Divalent ketoamine cross-link between two collagen molecules



Adapted from McCormick (1999)

Figure 1-5 Trivalent hydroxylysylpyridinoline cross-link



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Chapter 2

Modification of muscle inherent properties through age at slaughter, breed crosses, and growth promotants

2.1 INTRODUCTION

Beef producers have a myriad of production strategies to use to produce beef quickly and profitably. In Alberta, where 39% of the Canadian beef cattle and calf inventory is located (StatCan, 2010), cattle are usually slaughter processed at an average age of 18 months (AARD, 2009) and are usually British-Continental crossbreds (CBEF, 2009). Exogenous hormone implants are widely used to improve carcass meat yield and feed efficiency and the recent approval of the β-adrenergic agonist ractopamine hydrochloride for use in Canada adds yet another growth promotants available to beef producers.

Hormonal implants have been shown to improve final live weight and hot carcass weight of steers (Calkins et al., 1986; Perry et al., 1991; Platter et al., 2003; Roeber et al., 2000) and their mode of action is through growth hormone and insulin-like growth factor-I. Hormonal implants induce hypertrophy of muscle fibres (Ono et al., 1996) or a shift in muscle fibre type between fast glycolytic and fast oxidative glycolytic fibres (Fritsche et al., 2000). The β-adrenergic agonist ractopamine hydrochloride has been shown to increase the weight gain of steers (Avendaño-Reyes et al., 2006; Gruber et al., 2007; Winterholler et al., 2007) by binding to muscle β-adrenergic receptors. This provokes a shift in muscle fibre from slow oxidative to fast glycolytic and fast oxidative glycolytic (Gonzalez et al., 2009) as well as an enlargement of fast glycolytic and fast oxidative glycolytic muscle fibres (Strydom et al., 2009). Cooked beef toughness originates partly from the muscle fibre type and size (Crouse et al., 1991). Growth promotants cause changes in fibre pattern; therefore, they may also cause variation in meat tenderness. The economic return of using growth promotants to increase carcass weight is around $5 to $10 for each dollar spent on hormonal implants (AAF, 2008). This benefit may however

be counterbalanced by an increase in beef meat toughness, affecting the quality of the final meat product, potentially the consumer satisfaction, and eventual saleability.

Examination of various beef production strategies that are not commonly practiced may reveal economic advantages to producers and improvements in beef quality. Finishing steers at about 12 to 13 months of age could provide an economic advantage to producers by reducing production costs which would increase profit per beef animal if meat yield and quality are good. Also, the muscle fibre cross-sectional areas (Seideman et al., 1986; Wegner et al., 2000) and collagen insolubility (Hill, 1966) increase with age, and thus contribute to toughening beef meat (Møller, 1980). The connective tissue content and the muscle fibres may contribute less to meat toughness from a steer that is 12 to 13 months of age than from one that is 18 to 20 months of age. This could potentially improve the tenderness of beef muscle with moderate connective tissue content like the m. *gluteus medius*. Increasing the tenderness of moderate connective tissue muscles could add value to the carcass through marketing these muscles as eligible for grilling rather than braising.

This study investigated the effects of age at slaughter, breed crosses, hormonal growth implants, and ractopamine hydrochloride feed supplementation on the composition, fibre characteristics, and collagen characteristics of the m. *gluteus medius* and m. *semitendinosus* in order to identify meat quality advantages and disadvantages associated with various beef production practices.

2.2 MATERIALS AND METHODS

2.2.1 Experimental design and animal management

A 2 x 2 x 2 x 2 factorial experiment was designed to investigate the effect of age at slaughter, breed cross, hormonal growth implants, and feed supplementation with ractopamine hydrochloride on inherent properties of beef muscles. One hundred and twelve crossbred Hereford-Aberdeen Angus (HAA; n=64) or Charolais-Red Angus (CRA; n=48) steers born in April and May were identified with a tag and weighed about 24 hours after birth. Bull calves were castrated before two months of age. Calves and their dams were placed on meadow brome alfalfa pasture at the beginning of June. Calves

were weaned at an average age of 182 days with barley silage supplementation for 14 days prior to weaning. At weaning, calves were stratified by weight and assigned to either the calf-fed (n=56) or yearling-fed (n=56) finishing groups such that there were representatives of each breed cross within each system.

Steers in the calf-fed finishing group (calf-fed) were assigned to a hormonal growth implant group (NOIMP or IMP) with each breed cross represented in each treatment and groups equalized as much as possible for body weight. The 28 steers of the IMP group were implanted with Component E-S (200 mg progesterone and 20 mg estradiol benzoate) at about 200 days of age. During a 30 day adjustment period, steers were fed a grass hay diet and an increasing quantity of high barley grain ration. The final diet consisted of a 69.0% barley grain, 22.0% alfalfa-grass haylage, 8.4% feedlot supplement (32% crude protein (CP)), and 0.6% beet molasses (as fed basis; 1.24 kg/day; diet = total digestible nutrient (TDN) 76.7%, CP 13.8%). IMP steers were implanted with Component TE-S (120 mg trenbolone acetate and 24 mg estradiol) 83 days after first implantation. All steers were removed from the GrowSafe System pen about 30 days prior to slaughter and placed in feedlot pens (four NOIMP pens, four IMP pens), seven steers per pen with at least two steers of each crossbred type per pen. Half of NOIMP steers and half of IMP steers were assigned to the RAC treatment of 200 mg/head/day for 28 days of ractopamine hydrochloride (RAC; Optaflexx 45, Elanco Animal Health, Lacombe) assuming a dry matter intake (DMI) of 10 kg/head/day of a diet 92.8% barley grain and 7.2% Optaflexx 45, with each breed cross represented in each treatment and groups equalized as much as possible for body weight. Steers not fed RAC (NORAC) were kept on the finishing diet and all steers were finished to a constant back fat thickness of 8 to 9 mm as determined by ultrasound (Aloka 500V diagnostic real-time ultrasound with a 17 cm 3.5 Mhz linear array transducer (Overseas Monitor Corporation Ltd., Richmond, B.C)) using the procedure described by Brethour (1992).

The 56 steers assigned to the yearling-fed finishing group (yearling-fed) were divided into two hormonal growth implant groups, not implanted and implanted (NOIMP vs. IMP, respectively) with each breed cross represented in each treatment and groups equalized as much as possible for body weight. The 28 IMP steers were administered Component E-S at about 200 days of age and were kept grazing on meadow brome alfalfa pasture for 60 days. Steers were then placed in eight feedlot pens (four NOIMP pens, four

IMP pens), seven steers per pen with at least two steers of each crossbreed per pen, and fed the backgrounding diet consisting of 60% barley silage, 30% hay, and 10% barley:oat (60:40) rolled grain. IMP steers were re-implanted with Component E-S at about 280, 350, and 440 days of age. At 440 days, yearling-fed finishing steers were placed on meadow brome alfalfa pasture and removed after 62 days instead of 90 days due to drought and poor performance on pasture. Steers were placed into pens and fed 30 days with an adjustment diet of grass hay and an increasing amount of 21.2% barley silage, 74.1% rolled barley grain, 3.1% feedlot supplement (32% CP), and 1.6% molasses (as fed basis, TDN 80.2%; CP 13.1%). After the 30 day adjustment period, steers received the finishing diet (21.2% barley silage, 74.1% rolled barley grain, 3.1% feedlot supplement (32% CP), and 1.6% molasses; as fed basis, TDN 80.2%; CP 13.1%) and were implanted with Component TE-S. Steers were removed from the pens about 30 days prior to slaughter and placed in feedlot pens. Half of NOIMP steers and half of IMP steers were assigned to the RAC treatment with each breed cross represented in each treatment and groups equalized as much as possible for body weight. Steers were fed a diet containing 92.8% barley grain and 7.2% Optaflexx 45 to deliver 200 mg RAC/head/day assuming a DMI of 10 kg/head/day. NORAC steers were kept on the finishing diet. All steers were finished to a constant back fat thickness of 8 to 9 mm determined by ultrasound as described for the calf-fed steers.

2.2.2 Slaughter

Steers in the calf-fed finishing group were sent to the AAFC-Lacombe Research Center abattoir at 12 to 13 months of age and steers assigned to the yearling-fed finishing group were sent at 18 to 20 months of age. Two pens per kill (n=14 per kill) were sent to the abattoir the day before slaughter and steers were held overnight in lairage with free access to water. Live weight and steer identification were noted before stunning by captive bolt, and then exsanguination and carcass dressing proceeded in a simulated commercial manner. Carcasses were split and each half weighed and pasteurized. Following hot water pasteurization at 85°C for ten seconds, carcasses were chilled overnight at 2°C with wind speeds of five meter per second.

At 24 hour post mortem, the carcasses were fabricated and the left m. *semitendinosus* (ST; eye of round) and m. *gluteus medius* (GM; top sirloin) muscles

were removed. Muscles were individually labelled and weighed. Steaks were removed from the proximal to distal end for ST and from the anterior to posterior end for GM. The first trim steak was discarded and the second was cut 2.5 cm thick and used for muscle fibre type determination. The remaining muscle portion was weighed, vacuum-packaged, and aged at 4°C for seven days.

2.2.3 Fibre typing and cross-sectional area measurements

A muscle sample of about 0.5 cm x 0.5 cm x 1 cm was cut along the grain of the muscle fibres at about the centre of the steak. Samples were mounted separately on corks perpendicularly to the grain with Cryomatix (Thermo Shandon Inc., Pittsburgh PA), frozen in liquid nitrogen, and stored overnight at -35°C. Eleven µm thick muscle sections were removed at -20°C using a cryostat (Thermo Shandon Cryotome, Model 77210164 GB; Thermo Shandon Inc., Pittsburgh PA) and placed on a slide and frozen overnight in a freezer set at -35°C. Staining was performed according to the procedure of Solomon and Dunn (1988) with the acid pre-incubation and the ATPase solution made according to Guth and Samaha (1970) and the succinate dehydrogenase (SDH) staining according to Horák (1983).

Images for fibre typing and cross-sectional area measurement were captured with an Axioscope (Zeiss, West Germany) equipped with a Sony DXC 930 Color Video Camera (Sony Corporation, Japan). Measurement and typing analyses were performed on an Image Pro-Plus software V4.0 (Media Cybernetics, Silver Spring, MD). Muscle fibres were classified according to their speed of contraction and their oxidative and/or glycolytic capacities as slow oxidative (SO), fast oxidative glycolytic (FOG), or fast glycolytic (FG). Muscle fibre cross-sectional areas were averaged per type of fibre and expressed in μm^2, and muscle fibre distribution was calculated as the number of fibres of one type within the bundle divided by the total number of fibres in the bundle. For each sample, to have a good representation of the muscle, fibres of four bundles were typed and fibres of three bundles were measured.

2.2.4 Collagen characteristics

After seven days of ageing, a 2.5 cm thick steak was removed from each muscle. Steaks were weighed, labelled, vacuum-packaged, and frozen at -20°C until analysis. Prior to analysis, steaks were allowed to thaw at 4°C for 24 hours before being cut into 1 cm^3 pieces. At least 100 g per steak were weighed and placed in an aluminum pan to be lyophilised for 100 hours. After lyophilisation, samples were weighed, ground to a fine powder using a Waring blender (Model 7011C, Waring Commercial, Torrington, CT) and dry ice, and stored at -20°C until hydrolysis.

2.2.4.1 Total collagen content

The total collagen content was quantified by determination of the hydroxyproline content according to Bergman and Loxley (1963). Approximately 0.030 ± 0.005 g of the grind was transferred into a labelled glass tube. Five mL of 6 N HCl were added to each tube and a nitrogen draft was sent into the tube for about five seconds to remove oxygen. Tubes were then capped immediately after the nitrogen draft and hydrolysed for nine hours at 110°C (AccuBlockTM Digital Dry bath, Labnet International, Inc, Edison, NJ). After hydrolysis, tube contents were filtered using folded Whatman filter papers # 4 inserted in glass funnels. Each tube and filter paper were washed with approximately 5 mL of distilled water. Hydrolysates were stored in labelled 20 mL glass bottle overnight at 4°C.

The following day, samples were evaporated by rotary evaporation (Heidolph Collegiate rotary evaporator, Brinkmann, equipped with a DistiVac Ultra auto-purge vacuum system, Brinkmann, Mississauga, ON) at 60°C, with a rotation speed of 60 rpm and a vacuum pressure between 680-700 mm Hg. Once the evaporation was completed, the dry residue was reconstituted with 5 mL of distilled water. Samples were neutralized with 2.5 N and 0.2 N NaOH and pH was assessed using pH paper. A second rotary evaporation was performed on the samples with the same setting as the first. After evaporation, the dried residues were reconstituted with 5 mL of water. Two aliquots of 1 mL of each experimental sample were dispensed into labelled glass tubes with 2 mL of isopropanol and 1 mL of oxidant solution, which were mixed by swirling. Tubes were allowed to stand for 4 ± 1 minute before adding 13 mL of Ehrlich's reagent (100 g of p-

dimethylaminobenzaldehyde in 150 mL of perchloric acid (60%); further mixed with isopropanol in a 3:13 ratio, respectively). Tubes were capped, vortexed, and incubated in a 60°C water bath (Isotemp 228, Fisher Scientific, Ottawa, ON) for 25 minutes. Once the incubation time had elapsed, the tubes were cooled in an ice bath and kept in ice until analysis.

Following cooling, each tube was poured into a 50 mL volumetric flask and diluted to a final volume of 50 mL with isopropanol. Absorbance at 558 nm was measured ((Jasco V-630, Rose Scientific, Edmonton, AB) and (Evolution 60S, Thermo Scientific, Fisher Scientific, Ottawa, ON)) against a blank sample and a set of standard solutions with concentrations of 1.25, 2.5, 5, 10, and 20 µg hydroxyproline per mL, which were used to determine the hydroxyproline content. The factor 7.14 was used to convert the hydroxyproline content to the collagen content (Etherington and Sims, 1981). Total collagen was measured in duplicate from the beginning with each prepared sample assayed twice. The two collagen content values of one assay were averaged, and then the mean of both assays was calculated per muscle.

2.2.4.2 Soluble collagen content

Following the procedure of Hill (1966), 1.00 ± 0.01 g of lyophilized ground muscle was weighed into a centrifuge tube and 12 mL of ¼ strength Ringer's solution (2.25 g sodium chloride, 0.105 g potassium chloride, 0.159 g calcium chloride dehydrate, 1000 mL water) were added to the tube and the tube mixed using a vortex. Tubes were placed in a water bath (Isotemp 228, Fisher Scientific, Ottawa, ON) for 63 minutes at 77°C. After incubation in the water bath, each tube was centrifuged (Avanti J-E Centrifuge, Beckman Coulter, Mississauga, ON) for 20 minutes at 4000 x g and the supernatant was retained and placed in a labelled plastic bottle. Eight mL of Ringer's solution were added to the pellet, mixed using a vortex, and centrifuged a second time. The second supernatant was retained and pooled with the first one.

An aliquot of the supernatant (1.8 mL) was centrifuged (ManSci Mini Centrifuge, Mandel Scientific, Guelph, ON) for 25 seconds at 2000 x g and 1 mL of the liquid transferred into a glass tube. Three mL of 6 N HCl and 1 mL of 12 N HCL were added to each tube and the head space of each tube evacuated with nitrogen for about five seconds

to remove oxygen. Tubes were capped immediately after nitrogen evacuation and hydrolysed for nine hours at 110°C (AccuBlockTM Digital Dry bath, Labnet International, Inc, Edison, NJ). Steps after hydrolysis were the same as those for total collagen (Section 2.2.4.1) except no filtration was required after hydrolysis.

2.2.5 Proximate analysis

After seven days of ageing, 100 g of ground fresh muscle were oven dried at 102°C in a stainless steel beaker for 24 hours in a gravity convection-drying oven (VWR Scientific Model 1370FM; Mississauga, ON). The following day, beakers were removed from the oven and allowed to equilibrate at room temperature for ten minutes before final weights were recorded. Dried samples were crushed (Grindomix Model GM200, Retsch Inc., Newton, PA) and analyzed for fat content by petroleum ether extraction (Foss Soxtec System Model 2050; Foss Analytical AB, Hoganas, Sweden) (AOAC, 1995). Crude protein content determination was performed on fat-free samples (AOAC, 1997) with a Nitrogen/Protein Determinator CNS2000 (Leco Corp., St. Joseph, MI).

2.2.6 Statistical analysis

Data were analyzed as a 2 x 2 x 2 x 2 factorial design using PROC MIXED (SAS Version 9.2, SAS institute Inc., Cary, NC) with sources of variation including age at slaughter, implantation strategy, ractopamine hydrochloride feed supplementation, breed cross, and their two-, three-, and four-way interactions. Pen was included as a random effect. Denominator degrees of freedom were calculated using the Kenward-Roger approximation. For significant main or interaction effects ($P < 0.05$), differences between treatment or interaction means were computed using least square means and separated using *t*-tests with the PDIFF option.

2.3 RESULTS

2.3.1 Muscle weights

Results for muscle weights are presented in Tables 2-1 and 2-2 for the ST and the GM, respectively. Muscles were significantly heavier ($P < 0.0001$) in yearling-fed than in

calf-fed steers for both ST and GM muscles. IMP significantly increased the mean muscle weight of the GM ($P = 0.02$) and the ST ($P = 0.0006$). RAC affected neither the GM nor the ST mean muscle weights. The CRA crossbred steers had heavier GM ($P < 0.0001$) and ST ($P = 0.0003$) muscles than the HAA crossbred steers.

2.3.2 Proximate content of muscles

The proximate content of muscles is presented in Tables 2-1 to 2-3. Protein content of the ST was involved in a four-way interaction ($P = 0.04$) (Table 2-3). This four-way interaction appeared to be dominated by breed, RAC and implant differences, with IMP RAC CRA steers having greater mean muscle protein content than NOIMP NORAC HAA steers when yearling-finished. Although a four-way interaction was significant for ST mean protein content, main effects were examined as well due to the complexity of the interaction. ST, moisture and fat contents were not affected by age at slaughter, IMP, and RAC, but mean fat content tended to be higher ($P = 0.07$) in the muscles of NOIMP steers than in those of IMP steers. ST muscles from CRA steers had greater mean moisture ($P = 0.01$) and protein ($P < 0.0001$) contents and lower fat ($P < 0.0001$) content than those of HAA steers.

Four-way interactions were also significant in the GM for moisture ($P = 0.02$) and fat ($P = 0.04$) contents (Table 2-3). The mean GM moisture level was highest in RAC CRA yearling fed steers and lowest in RAC HAA calf-fed steers. RAC CRA yearling-fed steers had greater mean muscle moisture content than all calf-fed HAA steers and all non-implanted HAA yearling-fed steers. Although four-way interactions were significant for GM mean fat and moisture contents, main effects were examined as well due to the complexity of the interactions. GM protein tended to be reduced ($P = 0.07$) in yearling-fed steers compared to calf-fed steers without modification to the fat content. IMP decreased ($P < 0.0001$) fat and increased ($P < 0.0001$) moisture contents of the GM, while the CRA crossbred steers had increased protein ($P < 0.0001$) and moisture ($P = 0.02$) contents, and reduced ($P < 0.0001$) fat content. RAC had no effect on proximate content of the GM.

2.3.3 Muscle fibre characteristics

Treatment effects and those of their interactions on fibre cross-sectional areas are presented in Table 2-4 and Figures 2-1 to 2-3. Mean SO fibre cross-sectional areas in the ST were greater (P = 0.004) in muscles from carcasses of the yearling-fed than of the calf-fed steers and greater (P = 0.006) when steers were implanted than when not implanted. Mean SO fibre cross-sectional areas in the ST were not affected by RAC but there was a trend for mean SO fibre cross-sectional areas to be largest (P = 0.08) in HAA steers. IMP resulted in greater (P = 0.003) mean FOG fibre cross-sectional areas in the ST than the NOIMP FOG fibres. FOG fibre cross-sectional areas were involved in a three-way interaction where ST muscles from the carcasses of yearling-fed HAA steers had significantly greater (P = 0.03) mean FOG fibre cross-sectional areas than calf-fed HAA steers when not fed RAC. Conversely, CRA yearling-fed steers had greater mean FOG cross-sectional areas than CRA calf-fed steers when fed RAC (Figure 2-1). FG fibres in the ST were also involved in a three-way interaction where calf-fed CRA steers not fed with RAC had significantly greater (P = 0.01) mean FG cross-sectional areas than HAA calf-fed steers not supplemented with RAC (Figure 2-2). When they were fed with RAC, the ST muscles of yearling-fed CRA steers had larger mean FG cross-sectional areas than those of yearling-fed HAA steers supplemented with RAC. FG fibres of the ST were involved in a three-way interaction (Figure 2-3) in which muscles of carcasses from NORAC HAA steers of the IMP treatment had increased (P = 0.01) mean cross-sectional areas compared to those of the NORAC NOIMP HAA treatment. This difference was no longer significant when steers were fed RAC. On the other hand, ST muscles from carcasses of IMP and NOIMP NORAC CRA steers did not have significantly different mean FG cross-sectional areas, but when they were supplemented with RAC, ST muscles from carcasses of IMP CRA steers had greater mean FG cross-sectional areas than those of the NOIMP CRA steers, the NORAC NOIMP steers, and the NORAC IMP steers.

As for the GM, IMP and RAC did not affect fibre cross-sectional areas. Age at slaughter affected cross-sectional areas of SO and FOG fibres in the GM with yearling-fed steers having the greatest mean cross-sectional areas of both fibre types (P = 0.002 and P = 0.02, respectively). Mean FG cross-sectional areas were not affected by age at slaughter but the CRA breed cross increased (P = 0.05) their cross-sectional areas. Crossbreds did not affect the mean SO and FOG cross-sectional areas of the GM.

Fibre distributions are presented in Table 2-5 and their interactions in Figures 2-4 to 2-6. RAC did not affect the fibre distribution of the ST and moreover, SO fibre distribution of this muscle was not affected by any of the experimental treatments. Mean FG% increased (P = 0.0007) from calf-fed to yearling-fed steers while mean FOG% decreased (P = 0.002). Also, NOIMP HAA and CRA steers were similar for both FG and FOG fibre distribution in the ST; however, with IMP, HAA steer had lower (P = 0.02) mean FG% and higher (P = 0.002) mean FOG% than that of CRA steer (Figures 2-4 and 2-5).

In the GM, mean FG% of HAA and CRA steers were similar when calf-fed finished whereas mean FG% of muscles from the carcasses of HAA steer was lower (P = 0.04) than that of CRA steers when yearling-fed (Figure 2-6). In this same muscle, RAC supplementation increased mean FG% (P = 0.04) at the expense of the percentage of SO fibres (P = 0.02) without changing the proportion of FOG fibres. Mean SO% of the GM increased (P = 0.04) with IMP at the expense of FOG fibres (P = 0.04) while mean FG% remained stable. Mean SO% tended to be greater (P = 0.08) in yearling-fed steers than calf-fed steers in the GM and the CRA crossbred diminished (P = 0.04) SO%. Age at slaughter and breed cross did not affect FOG fibre distribution of the GM.

2.3.4 Collagen characteristics

Total and soluble collagen contents are presented in Tables 2-1 and 2-2 for the ST and the GM, respectively. IMP, RAC, and breed cross had no effect on the collagen contents in both the ST and GM. Total and soluble collagen contents of the ST were not affected by age at slaughter, although mean soluble collagen content tended to decrease (P = 0.06) in yearling-fed steers. For the GM, mean total collagen significantly increased (P = 0.02) with age at slaughter while mean soluble collagen decreased (P < 0.0001).

2.4 DISCUSSION

Muscle yield is of primary importance for beef producers because it partly determines their income, thus it is an economic concern that plays in the viability of their enterprise. In North America, cattle are grown for 16 to 20 months in order to achieve adequate carcass size without compromising meat tenderness. To enhance muscle yield

further, Continental breed cross, hormonal growth implants, and now β-adrenergic agonists (β-AA) are also used to increase feed efficiency and weight gain of cattle as well as yield grade. The effect of these production practices may be additive, and understanding how these strategies interact on muscles is necessary in order to interpret their effect on beef quality. Consumers may potentially not directly benefit from β-AA supplementation or implantation of steers, but because the cost of production is reduced with these strategies, it would be expected that they can have the same quality of meat for a reasonable price.

Within the present study, age at slaughter, IMP, and Continental breed cross increased ST and GM muscle weights. The increase in muscle weight with steer age appeared related to increases in mean SO and FOG fibre cross-sectional areas for both ST and GM. These results are not unexpected because, according to Jurie et al. (1995) and Wegner et al. (2000), cross-sectional areas of SO, FOG, and FG fibres should have increased between 12 and 18 months of age. Also in the ST, there was a shift in fibre type from FOG to FG fibres in the ST with increased steer age. Changes in fibre distribution of the ST with steer age disagreed with the findings of Wegner et al. (2000) who observed that changes in fibre frequency of German Angus, Galloway, Holstein Friesian, and Blue Belgian cattle occurred at a young age and remained consistent after six months of age. Jurie et al. (1995), however, observed a conversion of FOG to FG fibres in Limousin cattle until 12 months of age suggesting the results may be breed dependent. As well, results indicated that shifts in fibre type are muscle dependent, which may relate to the fibre distribution pattern of each individual muscle (Kirchofer et al., 2002) or the growth potential of different muscles at different times, as some muscles mature earlier than others with respect to lean body mass (Berg and Butterfield, 1976). Hence the results of the current study support the hypothesis that muscle fibres are not static entities and that their distribution can still vary at least until 18 to 20 months of age, depending on the breed cross, muscle, and production system.

The increase in muscle mass with IMP was associated with different changes to fibre type for each muscle as well. The mass increase in the GM was associated with an increased proportion of SO fibres at the expense of FOG fibres, while in the ST it was accompanied by increases in SO and FOG cross-sectional areas. Ono et al. (1996) also noted a differential response among muscles with hormonal implants and suggested that

growth occurred either by direct enlargement of fibres or by shifting from FOG to FG fibres. On the other hand, in the GM, the shift in fibre proportion from intermediate size FOG fibres to small size SO fibres with IMP does not suggest that the increase in muscle weight was necessarily due to fibre shifts, although mean SO cross-sectional areas tended to increase when the steer was implanted. The difference in fibre proportion was most likely due to the effects of the exogenous sex hormones because muscles from bulls are associated with reduced glycolytic fibre proportions due to their testicular hormones and higher muscle growth potential than steers (Lefaucheur and Gerrard, 2000). Indeed, lower occurrence of FG fibres and greater occurrence of SO fibres in bulls compared to steers were observed in the m. *longissimus dorsi* (Seideman et al., 1986), m. *semimembranosus*, and m. *semitendinosus* (Dreyer et al., 1977). Moreover, Young and Bass (1984) observed that the occurrence of FG fibres was lower in bulls than in steers, and their frequency was not correlated to the carcass weight of bulls and steers. Thus, it appears that natural and synthetic sex hormones promote an oxidative metabolism, but when lacking sex hormones, muscle metabolism shifts towards a glycolytic metabolism.

Causes of the changes in muscle weight associated with breed crosses were less obvious than those observed with hormonal implants and age at slaughter. Only a trend for smaller SO fibre cross-sectional areas in the ST muscle from CRA steers than HAA steers was observed, but ST muscle weight was heavier in CRA than in HAA steers. These observations strongly suggest that muscle weight was not dependent on fibre cross-sectional areas or distribution, but was rather due to either an increase in the total number of fibres (Rehfeldt et al., 2000) or a generalized growth of the muscle in which the muscle simply grew in length; however, these measurements were not taken. This is actually possible because the CRA crossbred will yield steers that have larger frames than those of the HAA crossbred (Buchanan and Dolezal, 1999). The ST is also a muscle involved in the movement of cattle as it is located in the hind limb (Sisson et al., 1975); accordingly, this muscle is stretched more than other muscles of the body (Gerrard et al., 1987).

Increase in GM muscle mass of CRA steers also seemed related to muscle fibre characteristics. Indeed, FG fibres were larger in the muscles of the carcasses from the CRA crossbred than in the HAA crossbred while the small size SO fibre proportion decreased, and unlike the ST, the GM is not extensively involved in movement (Sisson et al., 1975). So, basic biological differences such as the frame size of the cattle may affect

the size of the muscles, but more important, the biological functions of muscles may also affect their growth pattern. Therefore, the CRA crossbred offers the possibility of yielding more meat from one carcass than the HAA crossbred which definitely increases economic value for the beef producers. The cost of feeding and the gain:feed ratio of each breed type was not investigated in this study but are important considerations affecting profitability when choosing a breed.

Supplementation of the steers with RAC did not affect ST and GM muscle weights. An explanation for the lack of effectiveness of RAC may be related to in its selectivity for β1-adrenergic receptors (β-AR) (Hadley, 1996) and the abundance of this β-AR. β2-AR are the most abundant β-AR in cattle skeletal muscles (Sissom et al., 2007) while the β1-AR population is small (Sillence and Matthews, 1994). Therefore, β1-selective agonists have much less opportunity to bind to their receptors to transmit their action than β2-selective agonists. Also, β1-selective agonists such as RAC are less effective on cattle then β2-selective agonists (Moody et al., 2000). Although the results at the gross muscle level associated with RAC were not apparent, this growth promotant still induced changes in fibre distribution in the GM. Indeed, β-AA generally cause a shift of the myosin isoforms from a slow to a fast form (Polla et al., 2004), changing the contractile properties and energy metabolism of fibres from oxidative to glycolytic (Vestergaard et al., 1994). As observed with sheep in Aalhus et al. (1992), the FOG fibres shifted to FG fibres with RAC treatment. Interestingly, fibres of the GM shifted from SO to FG as they were expected to do according to Polla et al. (2004). This shift did not happen in the ST but may be due to the FG% of this muscle being much higher than in the GM. Even though RAC did not increase muscle weight, the changes observed in muscle fibres indicated there was an effect of RAC in muscles, but only at the muscle fibre level.

Changes in fibre distribution observed in the GM with RAC supplementation opposed those that were observed with IMP. Change in muscle fibres associated with RAC indicated a shift from oxidative to glycolytic metabolism, while IMP shifted muscle fibres towards an oxidative metabolism, confirming that these growth promotants induce muscle growth through different mechanisms. Moreover, although there was no interaction found between RAC and IMP on muscle weight, FG fibre cross-sectional areas of the ST were greatest when from muscles of CRA steers fed RAC and implanted. RAC is a β1-selective agonist and β1-AR are thought to be neuronal receptors (Hadley,

1996). Also, the neuronal effect of RAC and the hormonal effect of IMP can be additive but the effects of these two growth promotants on carcass characteristics have not been shown to be synergistic (Bass et al., 2009). Efficacy of RAC on the muscles studied was minimal because no improvement of muscle weight occurred when supplementing RAC. In other circumstances, RAC and IMP may interact more on steer muscle growth and carcass composition as observed in Baxa et al. (2010) with the β2-AA zilpaterol (ZH). This β-AA is however more efficient than RAC for improving steer growth and carcass yield (Strydom et al., 2009). Hormonal implants were thus overall more effective than RAC for inducing fibre changes and improving muscle weights in the muscles studied.

The interaction involving RAC, breed cross, and age at slaughter showed that FOG fibres of the ST muscle from the carcasses of calf-fed HAA steers were probably not fully mature at slaughter and had the capacity for further growth, which was observed with RAC supplementation. Also, results of the three-way interaction involving age at slaughter, RAC supplementation, and breed cross on ST FG fibre cross-sectional areas suggested that the early maturing, small framed HAA steers responded to RAC when supplemented early in their productive life. Conversely, the late maturing, large framed CRA steers responded to RAC when supplemented late in their productive life. Therefore, to maximize its effect, RAC has to be fed at the right time during growth, depending on the maturing profile of the steers.

Although four-way interactions were significant for the ST and GM proximate contents, main effects of treatments on the muscle proximate contents were examined rather than effects of the four-way interactions due to the complexity of interpreting four-way interactions. Hormonal implants were successful in decreasing the fat content of the GM. These results were not unexpected as improved weight gain and final weight and decreased intramuscular fatness are well established consequences of the administration of hormonal implants (Bruns et al., 2005). The greater fat content and the lower protein content observed in both the GM and ST muscles from the carcasses of HAA steers than from the carcasses of CRA steers were also expected because Angus and Hereford cattle have lower lean:fat ratio than Charolais cattle (Buchanan and Dolezal, 1999). Thus, intramuscular fat of muscle can be decreased through the use of hormonal implants and Continental breed such as Charolais.

Because consumers will be the ones eating the meat, it is important to satisfy their expectations, one of which is tender meat (Huffman et al., 1996). The tenderness of meat is partly determined by connective tissue (Purslow, 2005) and the GM and the ST have moderate and high amounts of collagen, respectively (Ramsbottom and Strandine, 1948). Although not compared statistically, total collagen content of the GM in yearling-fed steers seemed similar to that of the ST from both the calf-fed and yearling-fed steer carcasses. Also, the collagen solubility of the ST from calf-fed steer carcasses was lower than that of the GM from yearling-fed steer carcasses, which indicates the inherent difference in toughness due to connective tissue between these two muscles. A decline in the solubility of collagen is expected with increasing age because the number of insoluble cross-links increases with age (Shimokomaki et al., 1972). Collagen solubility in the ST tended to decrease with animal age at slaughter and was lowest at nine months of age and lower than the m. *infraspinatus* and m. *longissimus* (Gerrard et al., 1987). In this study, muscle collagen solubility decreased with age, but was not significant for the ST. The authors explained the great tension on ST collagen during muscle contraction decreased its solubility faster than in other muscles by increasing insoluble cross-link formation. Therefore, even though changes were observed in collagen in the GM, the contribution of connective tissue to meat toughness in the ST would most likely be greater than in the GM due to its increased resistance to heat. Connective tissue content was responsible for the poor tenderness score in the ST in the study of Shorthose and Harris (1990). These authors also said that, by prior myofibrillar tenderization of meat with tender-stretching or electrical stimulation, tenderness of the GM was acceptable until cattle were 48 month old, but the tenderness in the ST was not acceptable even when from steers younger than two years of age.

How the toughness of beef increases with age is well known (Shimokomaki et al., 1972), but this increase in toughness occurs only in muscles with moderate to high connective tissue content (Shorthose and Harris, 1990). Reducing the age at which beef cattle are slaughtered may reduce the contribution of collagen to meat toughness by shortening the period during which most of the heat-stable collagen cross-links would usually form. Therefore, because the solubility of collagen decreased with age in the GM, tenderness of this muscle may be improved by slaughtering steers at a young age. The results of the present study suggested that the ST may be still as tough in calf-fed steers as in yearling-fed steers because its collagen properties changed little with time, although

solubility tended to decrease with age. In contrast, because of its increased collagen solubility in carcasses from calf-fed steers, the GM may be a muscle that could be harvested as a premium tender muscle in young steers in order to obtain additional value from the beef carcass.

2.5 CONCLUSION

In the muscles studied, manipulation of steer age had the largest impact on the inherent properties of the meat. Hormonal growth implants were more effective than RAC at increasing muscle yield and inducing changes in muscle fibres but worked additively with RAC to increase mean FG fibre cross-sectional areas in Continental crossbred steers. Most importantly, the use of RAC had the greatest effect on muscle fibres of British crossbred steers when applied in a calf-finishing system and during yearling-finishing of Continental crossbred steers. Therefore, the maturity pattern of steers, and indeed of target muscles, should be taken into consideration when supplementing RAC. Slaughtering steers at 18 to 20 months of age, using hormonal growth implants, and using Continental crossbreds increased the yield of individual muscles. While age at slaughter significantly decreased collagen solubility in the GM, hormonal growth implants, RAC and breed crosses had no effect on collagen solubility in the muscles studied, suggesting that any effects on beef toughness by these production practices will be on muscle fibres.

2.6 TABLES

Table 2-1 Effect of age at slaughter, implantation strategy, ractopamine hydrochloride feed supplementation, and breed cross on inherent composition of the m. *semitendinosus*

Variable	Age at slaughter		Implantation		Ractopamine feeding		SEM[5]	Breed cross		SEM
	Calf-fed	Yearling-fed	NOIMP[1]	IMP[2]	NORAC[3]	RAC[4]		HAA[6]	CRA[7]	
n=	56	56	56	56	56	56	-	64	48	-
Muscle weight (g)	2192.53[b]	2745.88[a]	2282.00[b]	2656.41[a]	2414.26	2524.15	46.64	2357.95[b]	2580.45[a]	44.13
Moisture content (%)[8]	74.54	74.16	74.19	74.52	74.37	74.33	0.16	74.17[b]	74.53[a]	0.13
Fat content (%)	2.97	3.02	3.22	2.77	2.98	3.01	0.15	3.36[a]	2.63[b]	0.14
Protein content (%)	21.72	21.93	21.77	21.88	21.81	21.84	0.08	21.62[b]	22.03[a]	0.07
Total collagen (mg/g)	15.44	16.81	15.88	16.37	16.17	16.08	0.55	16.43	15.82	0.54
Soluble collagen (%)[9]	23.34	20.04	22.78	20.6	22.19	21.19	1.05	22.27	21.11	0.95

[1] No hormonal implant

[2] Hormonal implants

[3] No ractopamine hydrochloride feed supplementation

[4] Ractopamine hydrochloride feed supplementation

[5] Pooled standard error of the mean (SEM) for age at slaughter, implantation strategy, and ractopamine feed supplementation

[6] Crossbred Hereford-Aberdeen Angus

[7] Crossbred Charolais-Red Angus

[8] Summation of moisture, fat and protein contents within a treatment effect may not equal 100.00 due to number rounding

[9] Expressed as a percentage of total collagen content

[ab] Means within the same row within a main effect with different superscript letters are significantly different ($P < 0.05$)

Table 2-2 Effect of age at slaughter, implantation strategy, ractopamine hydrochloride feed supplementation, and breed cross on inherent composition of the m. *gluteus medius*

Variable	Age at slaughter		Implantation		Ractopamine feeding		SEM[5]	Breed cross		SEM
	Calf-fed	Yearling-fed	NOIMP[1]	IMP[2]	NORAC[3]	RAC[4]		HAA[6]	CRA[7]	
n=	56	56	56	56	56	56	-	64	48	-
Muscle weight (g)	3179.14[b]	4138.78[a]	3509.86[b]	3808.06[a]	3633.43	3684.49	73.24	3449.78[b]	3868.14[a]	64.13
Moisture content (%)[8]	73.50[b]	73.82[a]	73.34[b]	73.99[a]	73.62	73.71	0.11	73.48[b]	73.84[a]	0.11
Fat content (%)	3.35	3.23	3.71[a]	2.86[b]	3.24	3.33	0.12	3.69[a]	2.89[b]	0.12
Protein content (%)	22.03	21.73	21.77	21.99	21.92	21.84	0.10	21.65[b]	22.11[a]	0.09
Total collagen (mg/g)	11.98[b]	14.32[a]	13.02	13.28	12.95	13.36	0.56	13.56	12.74	0.50
Soluble collagen (%)[9]	52.31[a]	34.02[b]	43.01	43.32	43.33	43.00	2.13	42.62	43.71	2.13

[1] No hormonal implant

[2] Hormonal implants

[3] No ractopamine hydrochloride feed supplementation

[4] Ractopamine hydrochloride feed supplementation

[5] Pooled standard error of the mean (SEM) for age at slaughter, implantation strategy, and ractopamine feed supplementation

[6] Crossbred Hereford-Aberdeen Angus

[7] Crossbred Charolais-Red Angus

[8] Summation of moisture, fat and protein contents within a treatment effect may not equal 100.00 due to number rounding

[9] Expressed as a percentage of total collagen content

[ab] Means within the same row within a main effect with different superscript letters are significantly different ($P < 0.05$)

Table 2-3 Means of proximate analyses of the m. *gluteus medius* (GM) and m. *semitendinosus* (ST) with significant four-way interactions involving age at slaughter, implantation strategy, ractopamine hydrochloride feed supplementation, and breed cross

Muscle	Variable	Age at slaughter	NOIMP[1]				IMP[2]				SEM[7]
			NORAC[3]		RAC[4]		NORAC		RAC		
			HAA[5]	CRA[6]	HAA	CRA	HAA	CRA	HAA	CRA	
ST	Protein content (%)[8]	Calf-fed	21.52^{bcd}	21.83^{abcd}	21.44^{d}	21.94^{abce}	21.35^{de}	22.06^{abc}	21.65^{bcd}	21.95^{abcd}	0.20
		Yearling-fed	21.61^{cd}	22.13^{ab}	21.79^{abcd}	21.92^{abcd}	21.88^{abcd}	22.08^{abc}	21.75^{bcd}	22.29^{a}	
GM	Moisture content (%)	Calf-fed	73.05^{def}	73.42^{cdef}	72.74^{f}	73.77^{abcd}	73.58^{bcde}	74.34^{abc}	73.52^{bcdef}	73.58^{bcdef}	0.31
		Yearling-fed	72.87^{ef}	73.46^{bcdef}	73.71^{bcd}	73.70^{abcde}	74.32^{ab}	73.90^{abcd}	74.06^{abc}	74.59^{a}	
GM	Fat content (%)	Calf-fed	3.96^{abc}	3.26^{bcd}	4.52^{a}	3.12^{cde}	3.33^{bcd}	2.09^{f}	3.46^{bcd}	3.03^{cdef}	0.34
		Yearling-fed	4.34^{a}	3.24^{bcd}	4.11^{ab}	3.16^{bcd}	2.71^{def}	3.02^{cdef}	3.06^{cdef}	2.17^{ef}	

[1] No hormonal implant

[2] Hormonal implants

[3] No ractopamine hydrochloride feed supplementation

[4] Ractopamine hydrochloride feed supplementation

[5] Crossbred Hereford-Aberdeen Angus

[6] Crossbred Charolais-Red Angus

[7] Pooled standard error of the mean (SEM)

[8] Summation of moisture, fat and protein contents within a treatment effect may not equal 100.00 due to number rounding

^{abcdef} Means within each variable with different superscript letters are significantly different ($P < 0.05$)

Table 2-4 Effect of age at slaughter (A), implantation strategy (I), ractopamine hydrochloride feed supplementation (R), and breed cross (B) on fibre cross-sectional areas (μm^2) of the m. *semitendinosus* and m. *gluteus medius*, including significant interactions

Variable	Age at slaughter		Implantation		Ractopamine feeding		SEM[5]	Breed cross		SEM	Interaction	
	Calf-fed	Yearling-fed	NOIMP[1]	IMP[2]	NORAC[3]	RAC[4]		HAA[6]	CRA[7]		IxRxB	AxRxB
n=	56	56	56	56	56	56	-	64	48	-	-	-
M. *semitendinosus*												
SO[10]	2519.62[b]	3111.59[a]	2537.78[b]	3093.43[a]	2772.55	2858.66	107.47	2929.32	2701.88	100.06	NS[8]	NS
FOG[11]	3238.19[b]	3691.98[a]	3211.16[b]	3719.02[a]	3496.75	3433.43	83.30	3425.45	3504.73	81.73	NS	*[9]
FG[12]	4354.51	4784.63	4094.88[b]	5044.26[a]	4474.51	4664.62	194.71	4457.38	4681.75	156.06	*	*
M. *gluteus medius*												
SO	2471.93[b]	3119.56[a]	2632.63	2958.87	2794.00	2797.50	101.46	2830.05	2761.45	85.71	NS	NS
FOG	3016.99[b]	3558.14[a]	3167.59	3407.53	3312.17	3262.95	138.05	3299.29	3275.83	108.58	NS	NS
FG	4467.29	4889.84	4481.85	4875.29	4638.20	4718.94	201.71	4529.81[b]	4827.33[a]	160.64	NS	NS

[1] No hormonal implant
[2] Hormonal implants
[3] No ractopamine hydrochloride feed supplementation
[4] Ractopamine hydrochloride feed supplementation
[5] Pooled standard error of the mean (SEM) for age at slaughter, implantation strategy, and ractopamine feed supplementation
[6] Crossbred Hereford-Aberdeen Angus
[7] Crossbred Charolais-Red Angus
[8] Not significant
[9] Significant at ($P < 0.05$)
[10] Slow oxidative muscle fibre
[11] Fast oxidative glycolytic muscle fibre
[12] Fast glycolytic muscle fibre
[ab] Means within the same row within a main effect with different superscript letters are significantly different ($P < 0.05$)

Table 2-5 Effect of age at slaughter (A), implantation strategy (I), ractopamine hydrochloride feed supplementation (R), and breed cross (B) on fibre distribution[1] (%) of the m. *semitendinosus* and m. *gluteus medius*, including significant interactions

Variable	Age at slaughter		Implantation		Ractopamine feeding		SEM[6]	Breed cross		SEM	Interaction	
	Calf-fed	Yearling-fed	NOIMP[2]	IMP[3]	NORAC[4]	RAC[5]		HAA[7]	CRA[8]		AxB	IxB
n=	56	56	56	56	56	56	-	64	48	-	-	-
M. *semitendinosus*												
SO[11]	13.13	12.49	12.25	13.37	13.34	12.28	0.59	12.87	12.75	0.56	NS[9]	NS
FOG[12]	30.35[a]	23.72[b]	26.47	27.60	25.71	28.36	1.04	27.34	26.73	0.86	NS	*[10]
FG[13]	56.54[b]	63.78[a]	61.28	59.03	60.94	59.38	0.96	59.77	60.55	0.87	NS	*
M. *gluteus medius*												
SO	20.74	23.36	20.52[b]	23.58[a]	23.95[a]	20.15[b]	0.90	22.93[a]	21.18[b]	0.77	NS	NS
FOG	38.34	38.34	39.93[a]	36.74[b]	39.51	37.16	0.91	38.12	38.55	0.79	NS	NS
FG	40.89	38.32	39.54	39.67	36.55[b]	42.66[a]	1.69	39.04	40.16	1.31	*	NS

[1] Summation of SO, FOG, and FG % within a treatment effect may not equal 100.00 due to number rounding

[2] No hormonal implant

[3] Hormonal implants

[4] No ractopamine hydrochloride feed supplementation

[5] Ractopamine hydrochloride feed supplementation

[6] Pooled standard error of the mean (SEM) for age at slaughter, implantation strategy, and ractopamine feed supplementation

[7] Crossbred Hereford-Aberdeen Angus

[8] Crossbred Charolais-Red Angus

[9] Not significant

[10] Significant at ($P < 0.05$)

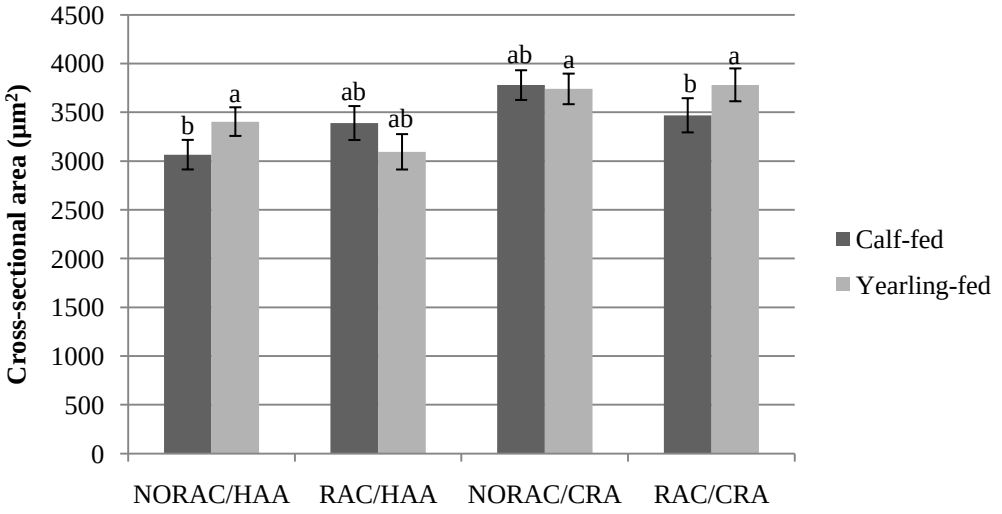
[11] Slow oxidative muscle fibre

[12] Fast oxidative glycolytic muscle fibre

[13] Fast glycolytic muscle fibre

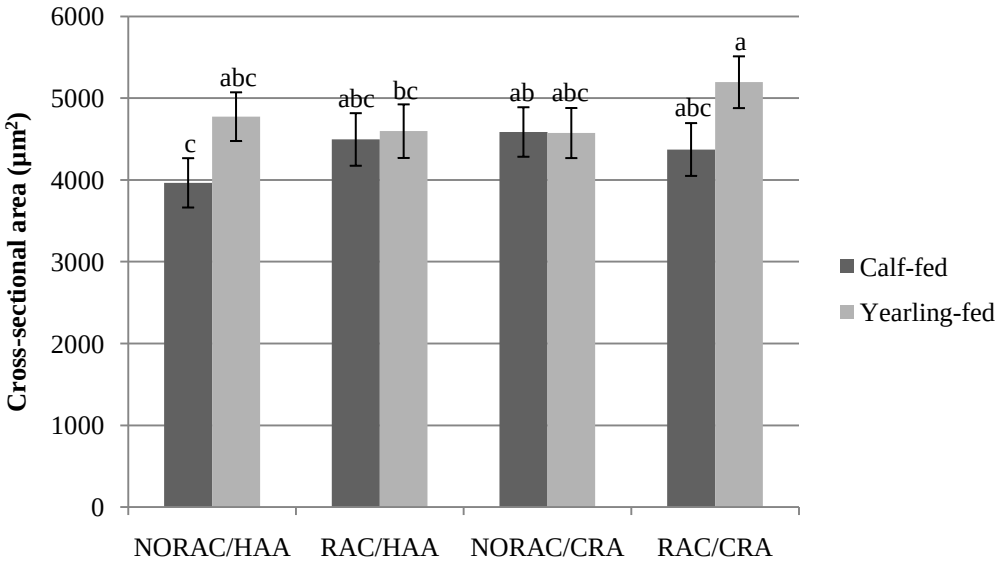
[ab] Means within the same row within a main effect with different superscript letters are significantly different ($P < 0.05$)

Figure 2-1 Fast oxidative glycolytic fibre cross-sectional areas of the m. *semitendinosus* as affected by an interaction between ractopamine hydrochloride feed supplementation, age at slaughter, and breed cross



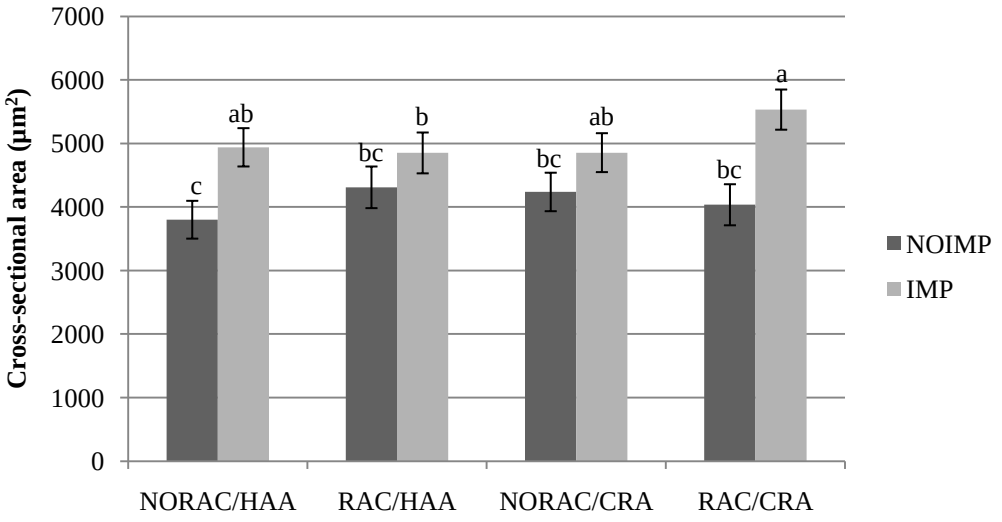
[ab] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
NORAC = No ractopamine hydrochloride feed supplementation; RAC = Ractopamine hydrochloride feed supplementation; HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus

Figure 2-2 Fast glycolytic fibre cross-sectional areas of the m. *semitendinosus* as affected by an interaction between ractopamine hydrochloride feed supplementation, age at slaughter, and breed cross



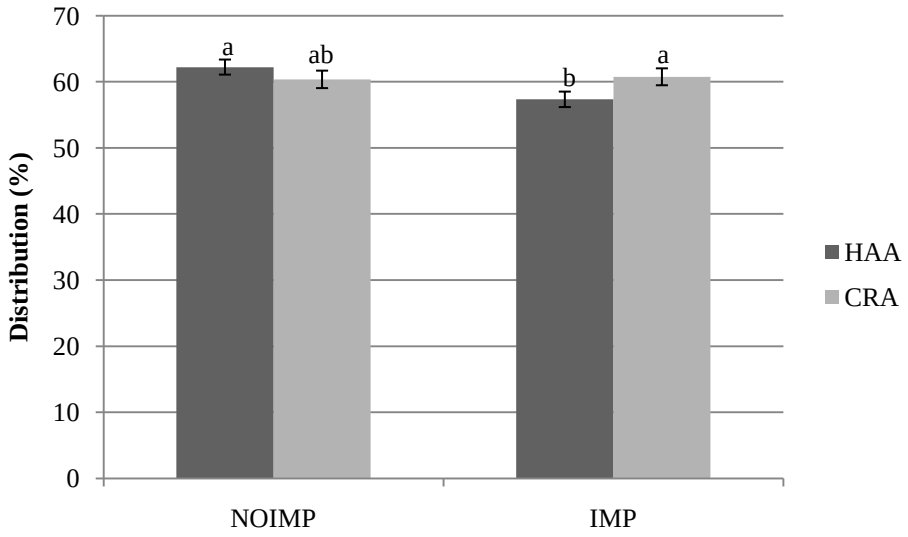
[abc] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
NORAC = No ractopamine hydrochloride feed supplementation; RAC = Ractopamine hydrochloride feed supplementation; HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus

Figure 2-3 Fast glycolytic fibre cross-sectional areas of the m. *semitendinosus* as affected by an interaction between ractopamine hydrochloride feed supplementation, implantation strategy, and breed cross



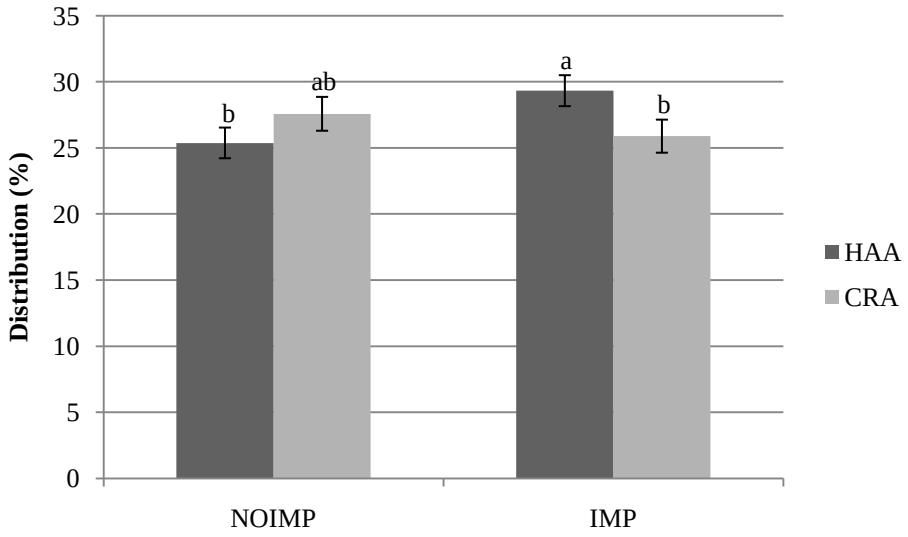
[abc] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
NOIMP = No hormonal implant; IMP = Hormonal implants; NORAC = No ractopamine hydrochloride feed supplementation; RAC = Ractopamine hydrochloride feed supplementation; HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus

Figure 2-4 Fast glycolytic fibre distribution of the m. *semitendinosus* as affected by an interaction between implantation strategy and breed cross



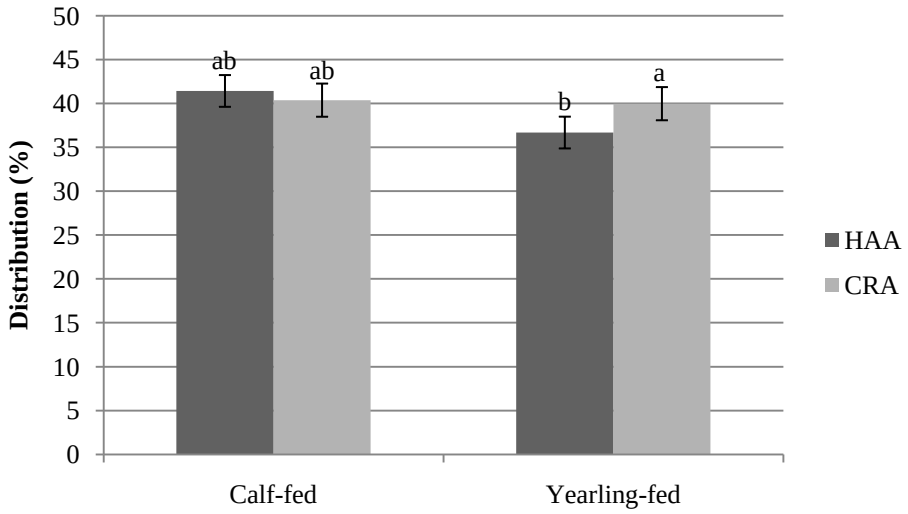
[ab] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus; NOIMP = No hormonal implant; IMP = Hormonal implants

Figure 2-5 Fast oxidative glycolytic fibre distribution of the m. *semitendinosus* as affected by an interaction between implantation strategy and breed cross



[ab] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus; NOIMP = No hormonal implant; IMP = Hormonal implants

Figure 2-6 Fast glycolytic fibre distribution of the m. *gluteus medius* as affected by an interaction between age at slaughter and breed cross



[ab] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus

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Chapter 3

Modification of meat quality through age at slaughter, breed crosses, and growth promotants

3.1 INTRODUCTION

A very important measurement to consider in meat quality is the eating satisfaction of the cooked product. In a survey by Huffman et al. (1996), tenderness was rated by consumers as the factor affecting their eating satisfaction the most. Tenderness is estimated by measuring the force required to shear or cut a cooked product and this measurement is performed with a mechanical device in order to represent the tenderness as evaluated by a taste panel. Ageing meat is a well-known process for tenderizing meat as proteolysis weakens myofibrils (Hopkins and Taylor, 2004; Ouali et al., 1987) and reduces the toughness of the muscle proteins (Pringle et al., 1993). Meat tenderization can vary over a long period of time, and has been shown to continue for up to 28 days post mortem (Gruber et al., 2006).

Muscle temperature, pH, colour, myoglobin state, and water-holding capacity are also characteristics widely measured in meat science to describe meat quality. They provide information on the chemical and physical changes occurring in muscles post mortem that can reflect the freshness and wholesomeness of meat (Troy and Kerry, 2010). For instance, the bright red colour of oxygenated myoglobin of meat is desired by consumers, but not the brownish colour that occurs as the myoglobin pigment oxidizes with time. At a certain point, quality measurements are also economic indicators because increased purge loss reduces the saleable product weight (Offer and Cousins, 1992), increases protein loss (Savage et al., 1990), and negatively impacts the buying choice of the consumers (Smith et al., 2000). Loss of colour stability in the retail case contribute to losses of $1.1 billion in the United States (NCBA, 1997) and $200 millions in Canada (Nattress and Yost, 1999).

Beef production strategies are usually formulated so that they have minimal effects on meat appearance and quality. Hormonal growth implants have no (Faucitano et al., 2008) or a slight effect on meat colour (Reiling and Johnson, 2003), and the β-adrenergic agonist ractopamine hydrochloride does not appear to affect meat colour (Gonzalez et al., 2009). Both hormonal implants (Faucitano et al., 2008; Foutz et al., 1997) and β-adrenergic agonists (Avendaño-Reyes et al., 2006; Gruber et al., 2008; Strydom et al., 2009) have been implicated in the toughening of beef and Australia recently price-penalized meat from cattle treated with growth promotants in its Meat Standards Australia guaranteed tender program (Thompson et al., 2008). The European Union also banned hormonal implants in 1988, restricting its meat importation to beef from cattle that did not receive hormonal implants (BSAS, 2010). As Canada seeks to add value to its beef through a Canadian Quality brand (BIC, 2010), examination of the effects of beef production strategies on the quality and composition of beef produced in Alberta has become a priority (ALMA, 2009).

Within a quality brand, using management systems that enhance the tenderness of meat could be economically beneficial for the beef producers. Indeed, reducing age at slaughter to 12 to 13 months of age increases collagen solubility (Section 2.3.4) and reduces muscle fibre cross-sectional areas (Section 2.3.3) which should reduce the toughness of beef (Shimokomaki et al., 1972; Unruh et al., 1986), but should also reduce production costs on the farm as cattle will be kept for a shorter time than the production system that keeps cattle on the farm until 18 to 20 months of age (AARD, 2009). Benefits from the increase in muscle weights resulting from the use of hormonal growth implants (CCA and BIC, 2001) are however in opposition to the reduction in beef tenderness (Foutz et al., 1997). Nevertheless, if the increase in beef toughness is minimized by certain production systems, the use of growth promotants may still be considered.

The effects of age at slaughter, breed crosses, hormonal growth implants, and ractopamine hydrochloride feed supplementation on the shear force, water-holding capacity, objective colour, myoglobin contents, and sarcomere length of m. *gluteus medius* and m. *semitendinosus* were investigated in order to evaluate the impact of various beef production practices on beef meat quality.

3.2.1 Experimental design and animal management

One hundred and twelve crossbred Hereford-Aberdeen Angus (HAA; n=64) or Charolais-Red Angus (CRA; n=48) were used in a 2 x 2 x 2 x 2 factorial experiment. Treatments included two ages at slaughter, 12 to 13 month of age, termed the calf-fed steers, and 18 to 20 month of age, the yearling-fed steers. Hormonal growth implants were also administered (IMP) or not (NOIMP), and ractopamine hydrochloride was supplemented (RAC) or not (NORAC). A complete description of animals and experimental treatments is described in Section 2.2.1.

3.2.2 Slaughter

Steers were sent to the AAFC-Lacombe Research Center abattoir (two pens per kill, n=14 per kill) the day before slaughter. Animals were held overnight in lairage with free access to water. Live weight and animal identification were noted before stunning by captive bolt, exsanguinating and dressing in a simulated commercial manner. Carcasses were split and each half weighed and pasteurized. Following hot water pasteurization at 85°C for ten seconds, carcasses were chilled overnight at 2°C with wind speeds of 5 m/sec.

At 24 hours post mortem, the carcasses were fabricated and the left m. *semitendinosus* (ST; eye of round) and m. *gluteus medius* (GM; top sirloin) muscles were removed. Muscles were individually labelled, weighed, and assessed for temperature and pH (Fisher Scientific Accumet AP72 pH meter [Fisher Scientific, Mississauga ON] equipped with an Orion Ingold electrode (Udorf, Switzerland)). Three readings for pH and one for temperature (T_{24h}) were recorded on each muscle with the mean pH calculated per muscle (pH_{24h}). Steaks were removed from the proximal to distal end for ST and from the anterior to posterior end for GM. The first trim steak was discarded and the second was cut 2.5 cm thick and used for sarcomere length and fibre type determination (Section 2.2.3). The remaining muscle portion was weighed, vacuum-packaged, and aged at 4°C for seven days.

3.2.3 Objective colour measurements, myoglobin relative contents, purge loss, cooking loss, and cooking time

Objective colour measurement and the proportion of myoglobin states were measured at 24 hours post mortem on the steaks used for sarcomere length. The steak was cut and allowed to stand for 20 minutes at 4°C to oxygenate the pigment (Boccard et al., 1981). Objective colour measurements were recorded three times per steak for lightness (L*), a* (red–green spectral axis), b* (yellow–blue spectral axis) (CIE, 1978) using a Minolta CR300 with Spectra QC-300 Software (Minolta Canada Inc., Mississauga, ON). Hue and chroma were determined as hue [H_{ab} = arctan(b*/a*)*57.296] and chroma [$C_{ab} = (a^{*2} + b^{*2})^{0.5}$]. Deoxymyoglobin (DMB), metmyoglobin (MMB), and oxymyoglobin (OMB) relative contents were determined based on reflex attenuance of incident light by interpolation of the isobestic points 473, 525, 572, and 730 nm at 24 hours post mortem (Krzywicki, 1979). Muscles were weighed, vacuum packaged, and aged at 4°C for seven days.

After ageing, muscles were weighed to determine purge loss, which was expressed in mg of water lost per g of muscle. Temperature and pH (pH_{7d}) were recorded as described for 24 hours measurements in Section 3.2.2. One 2.5 cm thick steak was cut and objective colour measurements were recorded as described for 24 hours with DMB, MMB, and OMB relative contents calculated. The steak was weighed, and a spear point temperature probe (Type T copper-constantan, 10 cm in length, AllTemp Sensors Inc., Edmonton, AB) was placed into its mid-point parallel to the longitudinal axis of the steak cross-section. Steaks were grilled at approximately 210°C (Garland Grill ED30B; Condon Barr Food Equipment Ltd., Edmonton, AB) with the internal temperature recorded at 30 second intervals. Steaks were turned when the internal temperature reached 35.5°C and cooked until the internal temperature reached 71°C (monitored with a Hewlett Packard HP34970A Data Logger; Hewlett Packard Co., Boise ID). As soon as 71°C was reached, cooked steaks were placed into polyethylene bags, sealed, cooled in an ice bath to prevent further cooking, and refrigerated overnight at 4°C. Cooking time was expressed in seconds required to cook one gram of raw steak. The following day, steaks were dried with filter paper to remove excess moisture and the weight of each was recorded to calculate cooking loss expressed in mg of water lost per g of raw steak.

3.2.4 Sarcomere length

Sarcomere length was measured as described by Aalhus et al. (1999). Two grams of muscle freed of fat and connective tissues were removed, scissor-minced, and mixed in 20 mL of a 0.02 M EGTA/0.25 M sucrose solution in a 50 mL centrifuge tube. Samples were homogenized for ten seconds at 6000 RPM (Polytron Homogenizer PT3100 and a 2 cm generator (Brinkmann Instruments Inc., Mississauga, ON, Canada)). One drop of each sample was placed on a slide with a cover slip for observation with an Axioscope (Zeiss, West Germany) equipped with a Sony DXC 930 Colour Video Camera (Sony Corporation, Japan). Three sarcomere lengths were measured per image with Image Pro-Plus software V4.0, (Mediacybernetics, Silver Spring, MD) and ten images were analysed per muscle sample. Lengths were averaged and expressed in µm.

3.2.5 Warner-Bratzler shear force

Six 1.9 cm diameter cores per steak were removed from cooked steaks parallel to the grain of the muscle fibres. Peak shear force (SF) was measured perpendicular to the muscle fibres using a Texture Analyser (Model TA.XT plus, Texture Technologies Corp, New York) equipped with a Warner-Bratzler shear head at a crosshead speed of 200 mm/min. Peak shear force was recorded in kg (Texture Exponent 32 Software, TextureTechnologies Corp., Hamilton, MA) and the six peak shear forces recorded per muscle were averaged and the standard deviation (STD) calculated.

3.2.6 Statistical analysis

Data were analyzed as a 2 x 2 x 2 x 2 factorial design with PROC MIXED (SAS Version 9.2, SAS institute Inc., Cary, NC) with sources of variation including age at slaughter, implantation strategy, ractopamine hydrochloride feed supplementation, breed cross, and their two-, three-, and four-way interactions. Pen was included as a random effect. Denominator degrees of freedom were calculated using the Kenward-Roger approximation. For significant main or interaction effects ($P < 0.05$), differences between treatment or interaction means were computed using least square means and separated using *t*-tests with the PDIFF option.

3.3 RESULTS

3.3.1 Temperature and pH

Muscle temperature and pH are presented in Tables 3-1 and 3-2 for the ST and GM muscles, respectively, and in Figure 3-1. Muscle T_{24h} was not affected by any of the treatments in either muscle. Mean muscle pH_{24h} of the ST was not affected by treatments; however, in the GM, mean muscle pH_{24h} was involved in a three-way interaction where RAC calf-fed HAA steers had a higher ($P = 0.02$) mean muscle pH than CRA steers of the same treatment (Figure 3-1). Mean muscle pH_{7d} of the ST tended ($P = 0.08$) to be greater in HAA steers than CRA steers, while in the GM, mean muscle pH_{7d} was significantly greater ($P = 0.003$) in HAA steers than in CRA steers.

3.3.2 Objective colour measurements

Results for objective colour measurements are presented in Tables 3-1 and 3-2 and Figure 3-2. Age at slaughter was the factor that affected raw muscle colour most. In the ST, raw steaks were significantly lighter ($P = 0.0007$), had greater ($P < 0.0001$) mean hue angle, and lower ($P = 0.02$) mean chroma values when from the calf-fed steer carcasses than from yearling-fed steer carcasses. Only mean hue angle increased ($P = 0.02$) with dietary supplementation of RAC in the ST, indicating an increase in yellow pigments. HAA meat tended to be lighter ($P = 0.09$) than CRA meat in the ST, but mean L* value of the GM was unchanged. In the GM, there was a tendency for lighter ($P = 0.06$) beef from calf-fed steers than yearling-fed steer carcasses, with greater ($P = 0.002$) mean hue angle and no change in mean chroma. In this same muscle, mean chroma was significantly higher ($P = 0.002$) in HAA than CRA steers when implanted and supplemented with RAC (Figure 3-2).

3.3.3 Myoglobin relative contents

Treatment effects and those of their interactions on mean muscle myoglobin relative contents are presented in Tables 3-1 and 3-2 and Figures 3-3 to 3-6. Mean MMB relative content was significantly higher for both the ST ($P = 0.02$) and the GM ($P = 0.0003$) from yearling-fed steer carcasses than from calf-fed steer carcasses. Mean

DMB and OMB relative contents were not altered by age at slaughter in the ST, but were involved in a three-way interaction in the GM where mean DMB relative content was greater (P = 0.04) in muscles from the carcasses of yearling-fed CRA steers supplemented RAC than those of HAA steers of the same age (Figure 3-3), and mean OMB relative content was greater (P = 0.03) in the muscles of carcasses from yearling-fed HAA steers supplemented RAC than those of CRA steers (Figure 3-4). Also, mean DMB and OMB relative contents of the GM were each involved in three-way interactions that included IMP, RAC, and breed types. In these interactions, when steers were implanted but not fed RAC, mean DMB relative content was greater (P = 0.004) in muscles of carcasses from HAA steers than those from CRA steers (Figure 3-5), whereas mean OMB relative content was greater (P = 0.003) in muscles of carcasses from CRA steers than from HAA steers of the same treatments (Figure 3-6).

3.3.4 Sarcomere length

Data for sarcomere length are presented in Tables 3-3 and 3-4 for ST and GM respectively. For both ST and GM muscles, sarcomere length was not affected by any main effects or their interactions.

3.3.5 Purge loss, cooking loss, and cooking time

Purge loss, cooking loss, and cooking time of beef steaks are presented in Tables 3-3 and 3-4 and Figure 3-7. Purge loss was greater (P = 0.02) for steaks from the ST muscles of yearling-fed than calf-fed steer carcasses, but cooking loss tended to be greater (P = 0.08), and cooking time was longer (P = 0.005) when steaks were from the ST muscles of calf-fed steer carcasses than when from those of yearling-fed steer carcasses. Purge loss was greater in ST steaks from carcasses of IMP treated steers than NOIMP steers (P = 0.01), and from carcasses of RAC fed steers than NORAC steers (P = 0.02). Cooking loss and cooking time of the ST were not affected by IMP and RAC treatments. HAA meat from ST muscles tended to have a smaller (P = 0.05) amount of purge loss, and to take longer (P = 0.09) to cook than meat from CRA steers. In the GM, purge loss was involved in a three-way interaction with IMP, RAC, and age at slaughter where purge loss was increased (P = 0.04) in steaks from the carcasses of calf-fed steer that received RAC while steaks from the carcasses of yearling-fed steers had increased

purge loss when treated with both RAC and IMP (Figure 3-7). Neither cooking loss nor cooking time were affected by age at slaughter, RAC, IMP, and breed cross in the GM, although there was a trend (P = 0.09) for cooking time to be lengthened in NORAC steers.

3.3.6 Peak shear force and its standard deviation

Peak shear force (SF) and standard deviation (STD) of SF results are presented in Tables 3-3 and 3-4 and Figures 3-8 and 3-9. In the ST, mean peak SF was involved in a two-way interaction and was greatest (P = 0.04) in steaks from the carcasses of yearling-fed steers of both crossbreds (Figure 3-8), but steaks from the HAA steer carcasses observed the greatest increase. Mean SF STD was also involved in a two-way interaction with age at slaughter and breed cross, and was lower (P = 0.02) in calf-fed HAA steer carcasses than those from all other treatments within this interaction (Figure 3-9). Implanting steers increased ST peak SF (P = 0.03) without affecting its STD, while RAC did not affect SF and STD of this muscle. Mean peak SF increased (P = 0.03) in the GM from the carcasses of yearling-fed steers and its STD tended to be greater (P = 0.07) than that from the carcasses of calf-fed steers. Peak SF varied with breed cross in the GM and was greater (P = 0.04) in muscles from CRA steers than those from HAA steers. Mean peak SF of the GM and its STD were not affected by IMP and RAC treatments.

3.4 DISCUSSION

3.4.1 Peak shear force

Of all the meat quality characteristics measured in meat science, SF is probably of the most interest because toughness is the most important factor affecting the appreciation of meat by consumers (Miller et al., 1995). Meat toughness is known to increase with age of steers (Shorthose and Harris, 1990); unfortunately, this means that the increase in the quantity of meat yielded in mature cattle will be at the expense of its tenderness. Hormonal growth implants also increase the final live weight and hot carcass weight of steers (Calkins et al., 1986; Perry et al., 1991; Platter et al., 2003; Roeber et al., 2000), and they have been implicated in meat toughening (Faucitano et al., 2008; Foutz et al., 1997), although not always (Cranwell et al., 1996; Kerth et al., 2003). Similarly the use of

RAC, which is considered a mild β-adrenergic agonist (β-AA), unlike clenbuterol and zilpaterol hydrochloride (ZH), has not always resulted in the toughening of meat (Strydom et al., 2009). Growth promotants can, therefore, be an alternative for increasing meat yield from carcasses in young steers as long as meat quality characteristics, especially tenderness, are not negatively affected.

Increases in cooked meat peak SF with animal age can originate from two fractions of the muscle: muscle fibres (Crouse et al., 1991) and connective tissue (Cross et al., 1973). Collagen, the main protein of connective tissues in skeletal muscles, can contribute to peak SF with its quantity but mostly with its heat insolubility (Boccard et al., 1979). Muscle fibres are the structural units of muscles and undergo proteolysis post mortem by proteases, calpains, cathepsins, and caspases (Houbak et al., 2008; Kemp et al., 2006; Nelson and Traub, 1983; Taylor et al., 1995). Calpain activity depends on their ante mortem level but also on the level of their inhibitor, calpastatin (Hopkins and Taylor, 2004). Indeed, as observed with lambs in Pringle et al. (1993), an increased concentration of calpastatin and a decreased content of μ-calpains post mortem resulted in increased peak SF, most likely due to a reduced rate of proteolysis ante mortem.

In the present study, age at slaughter was associated with increased peak SF of both the ST and the GM, and this increase was particularly marked in ST steaks from carcasses of both HAA and CRA steers. Collagen did not appear to contribute to tenderness variations with age in the ST because there was only a trend towards decreasing collagen solubility in yearling-fed steers (Section 2.3.4). Therefore, muscle fibres, and perhaps more precisely post mortem protein proteolysis, must be responsible for the increase in ST peak SF. The yearling-fed steers had a slow growth rate and thus may have had a lower protein turnover than calf-fed steers, which implies less degradation from proteases ante and post mortem (Koohmaraie et al., 2002), particularly in muscles that may have reached their mature size. Elastin content is high in the ST and may affect SF, but Cross et al. (1973) found no significant correlation between ST elastin content and SF. Elastin content in the muscles of the current study was not quantified. In the GM, the increase in peak SF was probably due to decreased collagen solubility as age at slaughter increased as the collagen solubility decreased from 52.31% to 34.02% over time (Section 2.3.4). Reduced post mortem proteolysis may have also been a factor contributing to the increase

in GM peak SF observed at the yearling-fed slaughter age as there was a toughening effect associated with the use of Continental crossbred.

IMP did not affect total and soluble collagen contents (Section 2.3.4), but did increase peak ST SF. The increase in peak SF associated with IMP use was most likely due to characteristics of the myofibrillar component of the muscle. The average cross-sectional area of fibres has been shown to contribute to the increase in peak shear force up to six days post mortem (Crouse et al., 1991). As measurements in the present study were taken at seven days post mortem, the increased cross-sectional areas of the fibres associated with hormonal implants use did not likely contribute to the increased peak SF. A reduction in post mortem proteolysis may account for the increased peak SF. The action of hormonal implants on muscle growth and protein deposition occurs through an increase in protease inhibitor activities rather than a decrease in the protease activities (Gerken et al., 1995). Protease and protease inhibitor activities were not measured in the present study, but based on Koohmaraie et al. (2002), it can be suggested that calpastatin and/or cystatin levels may have increased before death and remained at this high level after death. Therefore, they may have inhibited the proteases calpains and cathepsins ante and post mortem, resulting in lower post mortem proteolysis in IMP steers than in NOIMP steers. Consequently, hormonal growth implants increased muscle weight as expected but they had adverse effect on ST tenderness and their use to improve red meat yield in young steers should therefore be questioned if meat toughness is an issue. Ageing meat longer than seven days may however reduce the impact of hormonal implants on meat tenderness (Barham et al., 2003).

3.4.2 Purge loss and growth promotants

Purge loss is the water expelled from the myofibres during ageing and contributes, with cooking loss, to determine the overall water-holding capacity of meat. Purge loss is an economic concern as it reduces the saleable weight of meat (Offer and Cousins, 1992) and decreases the juiciness of meat (Van Oeckel et al., 1999). The nutritive value of meat is also affected because soluble protein is lost with purge (Savage et al., 1990). Industry prefers low purge losses; however, growth promotants like the β-AA ZH can increase drip loss, although RAC has not been shown to have this effect (Strydom et al., 2009). Therefore, even if growth promotants are used to enhance the profitability of beef

carcasses by improving muscle weight, they can have adverse effects and reduce the meat saleable weight.

The growth promotant RAC increased purge loss in both muscles, but the extent of effect differed between them. RAC consistently increased purge loss in the ST, as it was significant as a main effect, while RAC interacted with age at slaughter and implantation for purge in the GM. RAC appeared to be the most important factor in the interaction because the main effects for age at slaughter and hormonal implants on purge loss were not significant but that of RAC was. Increased drip loss is usually associated with higher L* values (Ryu and Kim, 2006) and protein denaturation (Penny, 1977). In the present study no change in any of the colour characteristics occurred with the main effect of RAC meaning that the altered purge loss with RAC did not appear to be related to protein denaturation. Muscles of the RAC treatment however had a shift from slow oxidative (SO) fibres to fast glycolytic (FG) fibres (Section 2.3.3) which may have contributed to increased purge loss. According to Geesink et al. (1993), FG fibres provide less support for water than SO fibres because FG fibres are larger and have a higher water:protein ratio than SO fibres. So, SO fibres would have less water and/or more protein than FG fibres relatively to the fibre size and proteins would bind more effectively water molecules in SO than FG fibres. Although this hypothesis was suggested for cooking loss and not purge loss, it can possibly be applied to purge loss as well because the mechanism of water retention in uncooked meat is also made through protein binding. As observed by Ryu and Kim (2006) with pork meat, the greatest water losses happened in meat having the greatest number of FG fibres, hypothetically due to an altered water:protein ratio. So, an alteration of the water:protein ratio with the increased number of large fibres could be responsible for the great purge loss in meat from RAC steers.

Although the water:protein ratio could be a path of explanation in the GM, purge loss in the ST and GM may not have the same cause. Indeed, fibre shifts occurred in the GM and the difference in water lost between the control and RAC treatments was 3.88 mg/g, but fibre shifts were not observed in the ST and the extent of purge loss between the treatments was reduced to 1.85 mg/g. In the ST, except the hue angle, no colour measurements or myoglobin relative contents varied. Hue angle has not been identified in the literature as a factor indicative of purge loss, but it may be indicative of changes happening in protein structures even though other colour measurements did not

significantly vary. Joo et al. (1999) studied the effect of different ultimate pH in pork meat and found that drip loss was greatest in muscle with a 'low' mean ultimate pH value of 5.34 compared to the mean ultimate pH values of 5.58, 5.74, and 6.47. The drip loss was also accompanied by increased values for L*, chroma, and hue angle. So, the hue angle can be associated with changes in muscle proteins and purge loss but is not usually the reference to indicate these changes, but rather L* is. Other factors such as the storage temperature, post mortem proteolysis, and the rate of pH decline can denature and/or affect the protein state (Lawrie and Ledward, 2006). Nevertheless, in the current study, storage conditions were the same, post mortem proteolysis, as shown with SF, was not affected by RAC, and the rate of pH decline was not measured. Otherwise, the increased purge loss in the ST due to RAC remains unexplained, but it is most likely that the mechanism causing the water loss is related to the proteins. This potential mechanism of purge loss could also have played a role in the water loss of the GM.

Purge loss was the greatest in the ST of implanted steers, with no change in any colour characteristics or relative myoglobin contents. This situation is similar to the one observed in the GM with significant purge loss in the RAC treatment. The most striking observation is that, once again, purge loss seemed related to the muscle fibres that were enlarged (Section 2.3.3) when steers were implanted, increasing the water:protein ratio and thus the ability of protein to hold water molecules. In the GM, purge loss was not affected by IMP and, interestingly, there was a shift from intermediate size fast oxidative glycolytic (FOG) fibres to small size SO fibres (Section 2.3.3), which most likely decreased the water:protein ratio.

Overall, the great purge losses associated with RAC and IMP reduced the saleable product weight and would be associated with the additional cost of feeding RAC or implantation, but may shift this cost into a different sector of the beef value chain. The results of this study suggest that if RAC is fed to yearling-fed cattle, purge loss can be minimized by not implanting steers compared to feeding RAC and implanting steers. . In calf-fed steers, implanting and feeding RAC to calf-fed steers can minimize purge loss compared to feeding RAC only. The use of RAC in Western Canadian beef steer production management should be approached with caution as RAC did not increase muscle growth (Section 2.3.1) and decreased the water-holding capacity of the ST and GM muscles in the present study. RAC fed to beef heifers in Western Canadian

production management systems may have similar effects than in beef steers but this remains to be proved. The use of hormone implants is equivocal as, even though they toughened meat and increased purge loss in the ST, muscle weights were improved. If growth promotants are to be used in bovine production, hormonal implants may be a better choice than RAC, but detrimental effects on meat quality may still occur.

3.4.3 Meat colour

Colour stability can be an indicator of shelf-life and wholesomeness of the meat product (Troy and Kerry, 2010). Moreover, the appearance and colour of meat is of paramount importance for consumers (Risvik, 1994) as it is used by consumers to indicate the freshness of a meat product (Boles and Pegg, 2005). The bright red colour of meat is more attractive than a brownish (Smith et al., 2000) or purplish colour (Carpenter et al., 2001). Myoglobin relative contents are good indicators of the red pigment of meat as the OMB gives its bright red colour to meat and MMB the brownish colour (Troy and Kerry, 2010). The stability of myoglobin is related to the availability of oxygen (Boles and Pegg, 2005) and is affected by several factors, one of which is the presence of aerobic bacteria. These bacteria reduce the concentration of oxygen and lead to the formation of MMB (Seideman et al., 1984) which is why consumers associate brownish meat with spoiled meat. Nevertheless, most loss of meat colour at retail is due to a lack of oxidative stability beyond three days of retail storage (Egan et al., 1988). The concentration of myoglobin in muscles also increases with age and makes meat from old steers darker than that from young steers (Seideman et al., 1984).

Age at slaughter was the factor affecting colour the most in the ST and the GM. Growth promotants and breed crosses had minimal effect on meat colour, if any. Both the GM and ST had significantly greater MMB content in carcasses from yearling-fed steers than in those from calf-fed steers, meaning that the appearance of meat may be browner in a yearling-fed steer than in a calf-fed steer. MMB content of forage-fed steers has been hypothesized to be greater than in grain-fed steers because of the level of exercise of steers on pasture (Muir et al., 1998), but other studies did not report this difference (O'Sullivan et al., 2003; Sapp et al., 1999). So, results of the current study may support the hypothesis that forage-based diets increase MMB muscle content, but diet treatments in the current study were confounded with age. The hue angle of beef from the calf-fed

steers indicated an increased amount of yellow pigment, which should have made the beef appear yellowier than in yearling-fed steers. In the ST, beef from the calf-fed steer carcasses was lighter and in the GM, beef tended to be lighter in calf-fed steers than yearling-fed steers, confirming the observation of Seideman et al. (1984). Increased beef lightness might improve the attractiveness of calf-fed beef to consumers; therefore, finishing beef at a young age may produce a meat product that better satisfies consumer's expectation for appearance and meat colour. Nevertheless, because meat was not evaluated in retail display by panellists, this assertion cannot be confirmed and whether the difference in meat colour due to age at slaughter observed in this study could be perceived by consumers was not explored.

3.5 CONCLUSION

Results indicated that reducing age at slaughter and excluding Continental breed crosses decreased cooked meat toughness of ST and GM steaks. Although RAC did not affect cooked muscle SF, its use in Western Canadian production systems is of concern, given that it did not increase muscle yield of youthful cattle and was associated with increased purge loss. SF of meat can be partly controlled by management practices used on the farm; therefore, production systems focussing on increasing beef yield should incorporate Continental crossbreds, hormone implants, and slaughter steers between 18 and 20 months of age. Producers seeking to reduce beef toughness should consider using British crossbreds, slaughter process steers at 12 to 13 months, and exclude hormonal implants.

3.6 TABLES

Table 3-1 Effect of age at slaughter, implantation strategy, ractopamine hydrochloride feed supplementation, and breed cross on meat pH, temperature, objective colour measurements, and myoglobin relative contents of the m. *semitendinosus*

	Age at slaughter		Implantation		Ractopamine feeding			Breed cross		
Variables	Calf-fed	Yearling-fed	NOIMP[1]	IMP[2]	NORAC[3]	RAC[4]	SEM[5]	HAA[6]	CRA[7]	SEM
n=	56	56	56	56	56	56	-	64	48	-
24 hours post mortem										
pH	5.66	5.67	5.67	5.66	5.69	5.64	0.04	5.67	5.66	0.03
Temperature (°C)	6.84	6.91	6.80	6.95	6.82	6.93	0.14	6.80	6.95	0.13
7 days post mortem										
pH	5.58	5.62	5.60	5.60	5.64	5.56	0.05	5.61	5.59	0.04
Lightness (L*)	47.80[a]	44.88[b]	46.29	46.39	46.15	46.53	0.38	46.64	46.04	0.33
Chroma (%)	25.04[b]	26.34[a]	25.58	25.8	26.00	25.38	0.32	25.84	25.54	0.27
Hue angle (°)	46.06[a]	42.58[b]	44.00	44.65	43.69[b]	44.95[a]	0.32	44.32	44.33	0.31
MMB[8,9]	0.12[b]	0.14[a]	0.13	0.14	0.13	0.13	0.00	0.14	0.13	0.00
DMB[10]	0.04	0.05	0.06	0.04	0.05	0.04	0.01	0.05	0.04	0.01
OMB[11]	0.83	0.81	0.81	0.83	0.81	0.83	0.01	0.82	0.82	0.01

[1] No hormonal implant
[2] Hormonal implants
[3] No ractopamine hydrochloride feed supplementation
[4] Ractopamine hydrochloride feed supplementation
[5] Pooled standard error of the mean (SEM) for age at slaughter, implantation strategy, and ractopamine feed supplementation
[6] Crossbred Hereford-Aberdeen Angus
[7] Crossbred Charolais-Red Angus
[8] Summation of MMB, DMB, and OMB relative contents within a treatment effect may not equal 1.00 due to number rounding
[9] Metmyoglobin relative content
[10] Deoxymyoglobin relative content
[11] Oxymyoglobin relative content
[ab] Means within the same row within a main effect with different superscript letters are significantly different ($P < 0.05$)

Table 3-2 Effect of age at slaughter (A), implantation strategy (I), ractopamine hydrochloride feed supplementation (R), and breed cross (B) on meat pH, temperature, objective colour measurements, and myoglobin relative contents of the m. *gluteus medius*, including significant interactions

	Age at slaughter		Implantation		Ractopamine feeding			Breed cross			Interaction	
Variables	Calf-fed	Yearling-fed	NOIMP[1]	IMP[2]	NORAC[3]	RAC[4]	SEM[5]	HAA[6]	CRA[7]	SEM	IxRxB	AxRxB
n=	56	56	56	56	56	56	-	64	48	-	-	-
24 hours post mortem												
pH	5.63	5.58	5.61	5.60	5.63	5.58	0.03	5.62	5.59	0.03	NS[8]	*[9]
Temperature (°C)	6.59	6.63	6.61	6.60	6.48	6.74	0.22	6.70	6.52	0.19	NS	NS
7 days post mortem												
pH	5.57	5.57	5.57	5.57	5.61	5.53	0.06	5.58[a]	5.56[b]	0.04	NS	NS
Lightness (L*)	41.38	39.26	39.69	40.95	40.42	40.23	0.68	40.86	39.79	0.56	NS	NS
Chroma (%)	27.83	29.39	28.41	28.82	28.91	28.31	0.59	28.99[a]	28.23[b]	0.45	*	NS
Hue angle (°)	37.43[a]	35.72[b]	36.27	36.87	36.47	36.67	0.27	36.72	36.42	0.23	NS	NS
MMB[10,11]	0.17[b]	0.19[a]	0.18	0.18	0.18	0.18	0.00	0.18	0.18	0.00	NS	NS
DMB[12]	0.08	0.06	0.07	0.07	0.07	0.07	0.01	0.07	0.07	0.01	*	*
OMB[13]	0.75	0.75	0.75	0.75	0.75	0.75	0.01	0.75	0.75	0.01	*	*

[1] No hormonal implant

[2] Hormonal implants

[3] No ractopamine hydrochloride feed supplementation

[4] Ractopamine hydrochloride feed supplementation

[5] Pooled standard error of the mean (SEM) for age at slaughter, implantation strategy, and ractopamine feed supplementation

[6] Crossbred Hereford-Aberdeen Angus

[7] Crossbred Charolais-Red Angus

[8] Not significant

[9] Significant at ($P < 0.05$)

[10] Summation of MMB, DMB, and OMB relative contents within a treatment effect may not equal 1.00 due to number rounding

[11] Metmyoglobin relative content

[12] Deoxymyoglobin relative content

[13] Oxymyoglobin relative content

[ab] Means within the same row within a main effect with different superscript letters are significantly different ($P < 0.05$)

Table 3-3 Effect of age at slaughter (A), implantation strategy, ractopamine hydrochloride feed supplementation, and breed cross (B) on water-holding capacity, cooking time and shear force of the m. *semitendinosus*, including significant interactions

	Age at slaughter		Implantation		Ractopamine feeding			Breed cross			Interaction
Variables	Calf-fed	Yearling-fed	NOIMP[1]	IMP[2]	NORAC[3]	RAC[4]	SEM[5]	HAA[6]	CRA[7]	SEM	AxB
n=	56	56	56	56	56	56	-	64	48	-	-
Sarcomere length (µm)	1.87	1.88	1.88	1.87	1.82	1.93	0.05	1.90	1.85	0.04	NS[8]
Purge loss (mg/g)	11.21[b]	12.94[a]	11.10[b]	13.05[a]	11.15[b]	13.00[a]	0.44	11.51	12.64	0.42	NS
Cooking loss (mg/g)	277.8	253.62	258.53	272.9	265.02	266.4	8.18	265.65	265.77	7.312	NS
Cooking time (sec/g)	7.12[a]	4.87[b]	6.48	5.50	5.80	6.19	0.41	6.34	5.65	0.35	NS
Shear force (kg)	6.47[b]	8.31[a]	6.92[b]	7.87[a]	7.25	7.53	0.26	7.37	7.41	0.21	*[9]
STD[10]	1.11[b]	1.34[a]	1.17	1.28	1.24	1.21	0.07	1.20	1.26	0.08	*

[1] No hormonal implant

[2] Hormonal implants

[3] No ractopamine hydrochloride feed supplementation

[4] Ractopamine hydrochloride feed supplementation

[5] Pooled standard error of the mean (SEM) for age at slaughter, implantation strategy, and ractopamine feed supplementation

[6] Crossbred Hereford-Aberdeen Angus

[7] Crossbred Charolais-Red Angus

[8] Not significant

[9] Significant at ($P < 0.05$)

[10] Standard deviation of shear force

[ab] Means within the same row within a main effect with different superscript letters are significantly different ($P < 0.05$)

Table 3-4 Effect of age at slaughter, implantation strategy, ractopamine hydrochloride feed supplementation, and breed cross on water-holding capacity, cooking time and shear force of the m. *gluteus medius*

Variables	Age at slaughter		Implantation		Ractopamine feeding			Breed cross		
	Calf-fed	Yearling-fed	NOIMP[1]	IMP[2]	NORAC[3]	RAC[4]	SEM[5]	HAA[6]	CRA[7]	SEM
n=	56	56	56	56	56	56	-	64	48	-
Sarcomere length (µm)	1.64	1.71	1.68	1.67	1.61	1.74	0.05	1.68	1.67	0.04
Purge loss (mg/g)[8]	13.5	13.51	13.65	13.35	11.56[b]	15.44[a]	0.56	13.54	13.46	0.51
Cooking loss (mg/g)	220.66	224.59	216.05	229.21	225.69	219.57	7.50	219.63	225.62	6.30
Cooking time (sec/g)	2.43	2.25	2.38	2.30	2.48	2.20	0.10	2.31	2.37	0.10
Shear force (kg)	5.29[b]	6.37[a]	5.59	6.07	5.71	5.94	0.28	5.64[b]	6.01[a]	0.22
STD[9]	1.06	1.27	1.08	1.25	1.19	1.14	0.07	1.21	1.12	0.07

[1] No hormonal implant

[2] Hormonal implants

[3] No ractopamine hydrochloride feed supplementation

[4] Ractopamine hydrochloride feed supplementation

[5] Pooled standard error of the mean (SEM) for age at slaughter, implantation strategy, and ractopamine feed supplementation

[6] Crossbred Hereford-Aberdeen Angus

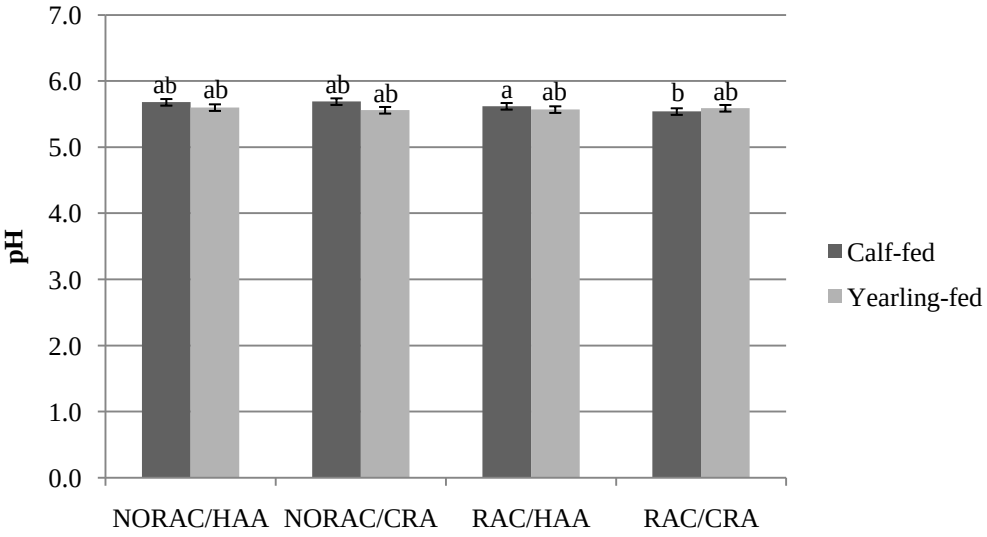
[7] Crossbred Charolais-Red Angus

[8] Purge loss was involved in a three-way interaction presented in Figure 3-7

[9] Standard deviation of shear force

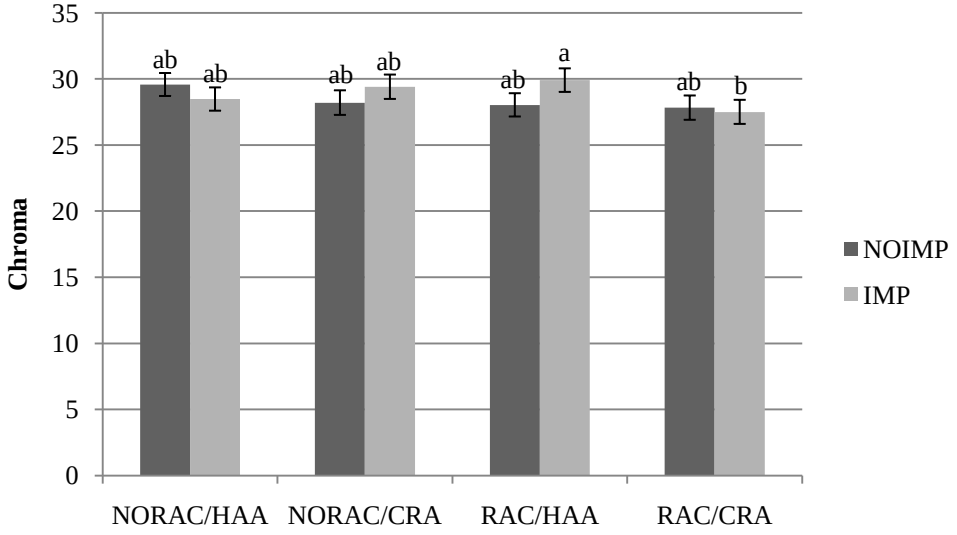
[ab] Means within the same row within a main effect with different superscript letters are significantly different ($P < 0.05$)

Figure 3-1 Mean pH 24 hours of the m. *gluteus medius* as affected by an interaction between ractopamine hydrochloride feed supplementation, age at slaughter, and breed cross



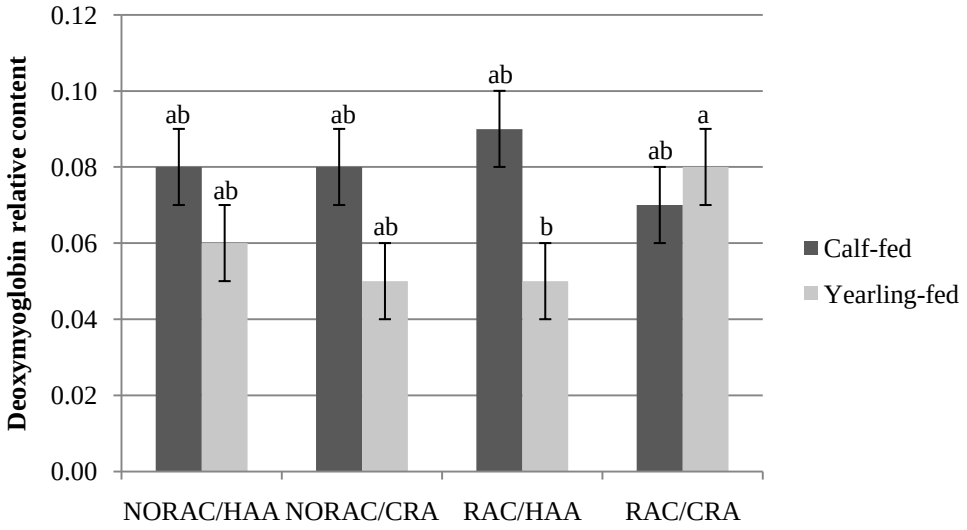
[ab] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
NORAC = No ractopamine hydrochloride feed supplementation; RAC = Ractopamine hydrochloride feed supplementation; HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus

Figure 3-2 Mean chroma value of the m. *gluteus medius* as affected by an interaction between ractopamine hydrochloride feed supplementation, implantation strategy, and age at slaughter



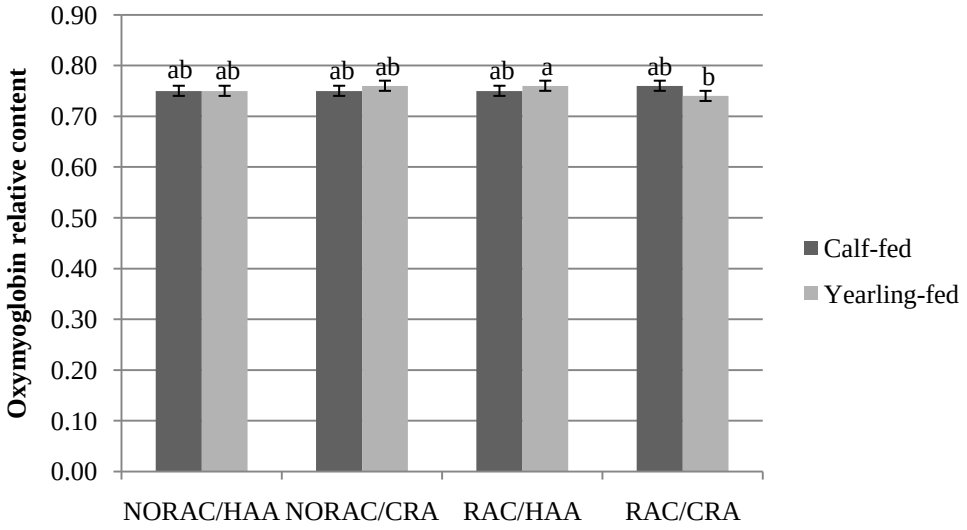
[ab] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
NOIMP = No hormonal implant; IMP = Hormonal implants; NORAC = No ractopamine hydrochloride feed supplementation; RAC = Ractopamine hydrochloride feed supplementation; HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus

Figure 3-3 Mean deoxymyoglobin relative content of the m. *gluteus medius* as affected by an interaction between ractopamine hydrochloride feed supplementation, age at slaughter, and breed cross



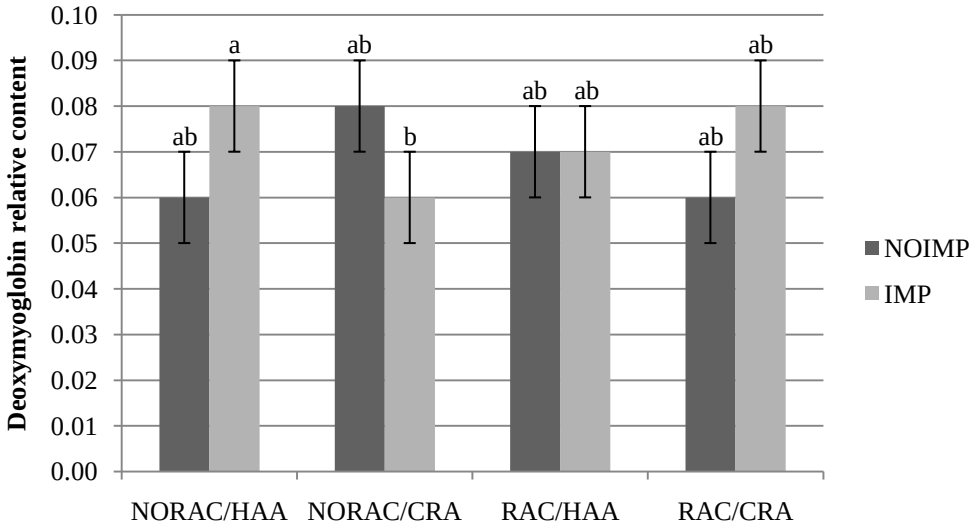
[ab] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
NORAC = No ractopamine hydrochloride feed supplementation; RAC = Ractopamine hydrochloride feed supplementation; HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus

Figure 3-4 Mean oxymyoglobin relative content of the m. *gluteus medius* as affected by an interaction between ractopamine hydrochloride feed supplementation, age at slaughter, and breed cross



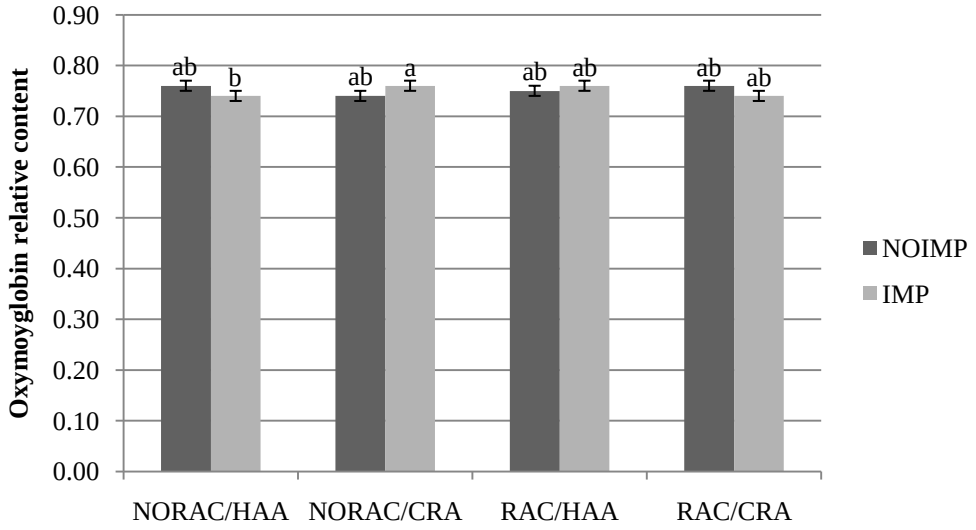
[ab] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
NORAC = No ractopamine hydrochloride feed supplementation; RAC = Ractopamine hydrochloride feed supplementation; HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus

Figure 3-5 Mean deoxymyoglobin relative content of the m. *gluteus medius* as affected by an interaction between ractopamine hydrochloride feed supplementation, implantation strategy, and age at slaughter



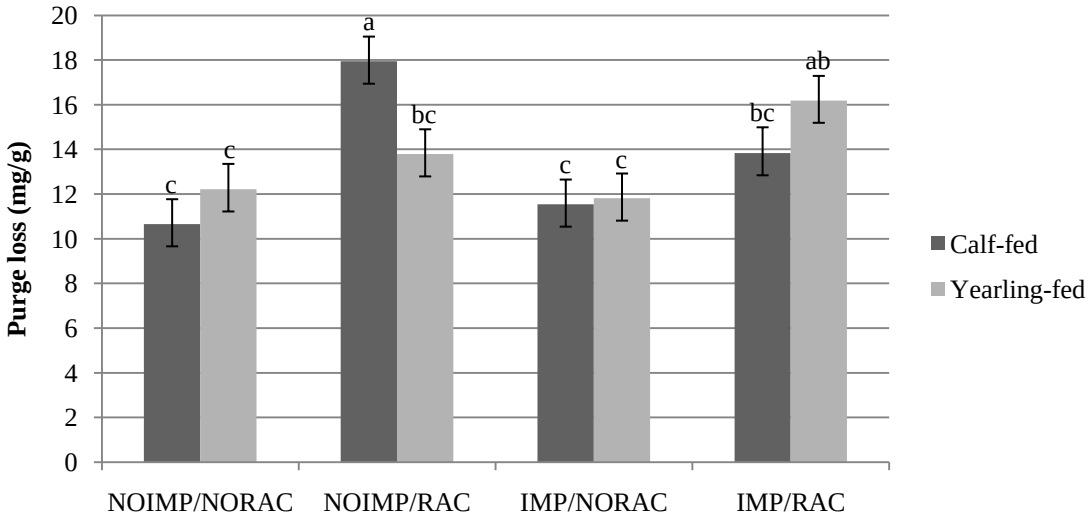
[ab] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
NOIMP = No hormonal implant; IMP = Hormonal implants; NORAC = No ractopamine hydrochloride feed supplementation; RAC = Ractopamine hydrochloride feed supplementation; HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus

Figure 3-6 Mean oxymyoglobin relative content of the m. *gluteus medius* as affected by an interaction between ractopamine hydrochloride feed supplementation, implantation strategy, and age at slaughter



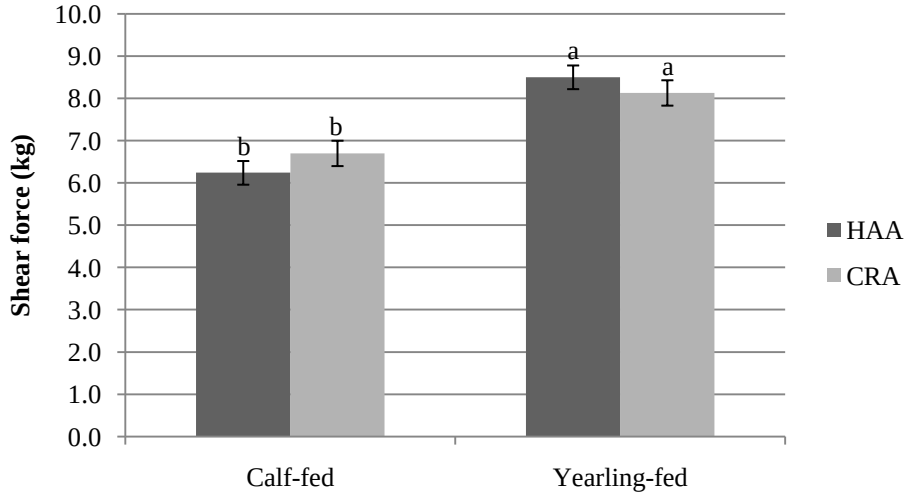
[ab] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
NOIMP = No hormonal implant; IMP = Hormonal implants; NORAC = No ractopamine hydrochloride feed supplementation; RAC = Ractopamine hydrochloride feed supplementation; HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus

Figure 3-7 Purge loss of the m. *gluteus medius* as affected by an interaction between ractopamine hydrochloride feed supplementation, implantation strategy, and age at slaughter



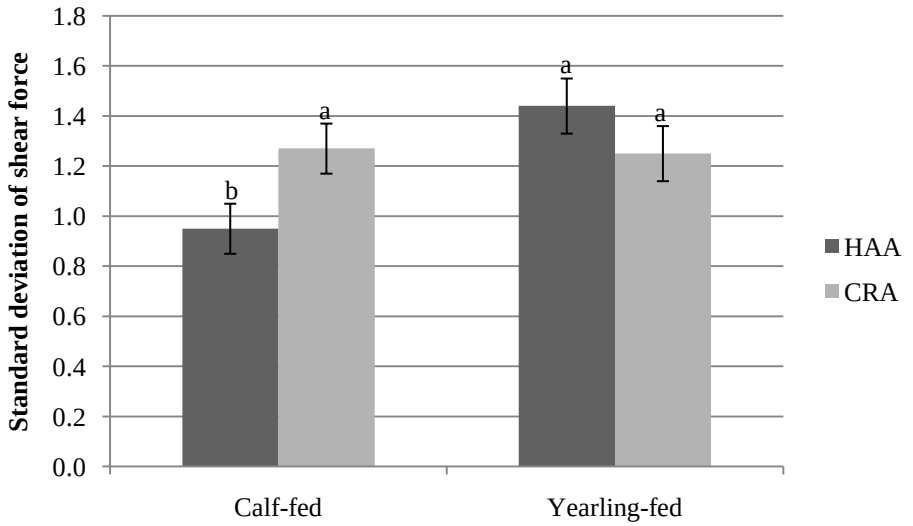
[abc] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
NOIMP = No hormonal implant; IMP = Hormonal implants; NORAC = No ractopamine hydrochloride feed supplementation; RAC = Ractopamine hydrochloride feed supplementation

Figure 3-8 Shear force of the m. *semitendinosus* as affected by an interaction between age at slaughter and breed cross



[ab] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus

Figure 3-9 Standard deviation of shear force of the m. *semitendinosus* as affected by an interaction between age at slaughter and breed cross



[ab] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus

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Chapter 4

Analysis of the Warner-Bratzler shear force with myofibrillar and connective tissue components

4.1 INTRODUCTION

Warner-Bratzler shear force is a technique that has been used since the 1930's to estimate the toughness of raw and cooked meat (Bratzler, 1958). Initially it was used to indicate the greatest amount of force or peak force required to cut meat, and the addition of recording devices has evolved the Warner-Bratzler shear apparatus into a device that produces a deformation curve showing either force exerted over time or force exerted versus the distance the cutting blade has travelled. Deformation curve graphs generated when measuring shear force with a Warner-Bratzler device have been studied in the past in an attempt to understand the causes of meat toughness and to partition shear force. Indeed, Bouton et al. (1975a) studied the relationship between different myofibrillar contraction states and the tensile and adhesion properties of meat. Møller (1980) examined the appearance of the shear deformation curve and how it related to the myofibrillar and connective tissue components of tenderness. Recently, Pietrasik et al. (2009) looked at the effect of moisture enhancement and pancreatin enzyme treatment on the myofibrillar and connective tissue components of meat and the subsequent shear force deformation curves.

Two main components of meat, myofibres and connective tissue, are considered to be the main determinants of the shear force deformation curve (Møller, 1980). A shear force curve always has a maximum positive peak shear force, but peaks of less importance are also, but not always, observed before and after maximum positive peak shear force (Figure 4-1). The small peaks occurring before maximum peak shear force are related to the myofibrillar component of shear force, whereas the ones after maximum peak shear force are due to the connective tissue that stretches before breaking (Møller,

1980). According to Bouton et al. (1975b), the Warner-Bratzler shear force indicates the myofibrillar component better than the connective tissue component of shear force.

The myofibrillar component, with the myofibrillar structure (Bouton et al., 1981) and contraction state of muscle (Bouton et al., 1975b), represents the muscle fibres (Møller, 1980) and their sarcomere lengths (Bouton et al., 1975b; Bouton et al., 1974). The myofibrillar component includes variations due to muscle fibre composition type and size (Kirchofer et al., 2002), both of which can vary with animal age (Jurie et al., 1995; Wegner et al., 2000) and breed (Johnston et al., 1975; Wegner et al., 2000). It also includes the effect of sarcomere length, which is affected by factors such as the early post mortem glycolytic rate of muscles (O'Halloran et al., 1997), their ultimate pH (Silva et al., 1999), post mortem ageing temperature (Devine et al., 2002), carcass position and suspension (Herring et al., 1965; Hostetler et al., 1972). On the other hand, the connective tissue component represents the connective tissue network (Bouton et al., 1975b; Bouton et al., 1981) and encompasses the total collagen content and its solubility (Møller, 1980). Muscles have different collagen contents (Bendall, 1967), with collagen solubility varying with animal age (Gerrard et al., 1987; Herring et al., 1967; Hill, 1966) and breed (Chambaz et al., 2003). By identifying the myofibrillar and the connective tissue shear forces within a shear force deformation curve, we may be able to relate muscle composition variations to changes in shear force of this muscle, and then associate the variation with specific production treatments applied to cattle. This would assist with understanding which meat quality variables are the most effective at predicting shear force. Developing an equation to predict maximum peak shear force could also help elucidate the mechanisms affecting shear force and give information on whether the myofibres or the connective tissue is the main factor contributing to shear force.

To address this need, the aim of this study was to investigate if shear force can be separated into connective tissue and myofibrillar components, to determine if these components were related to muscle and meat characteristics, and to examine if these characteristics could help predict shear force of cooked beef meat.

4.2.1 Experimental design and animal management

One hundred and twelve crossbred Hereford-Aberdeen Angus (HAA; n=64) or Charolais-Red Angus (CRA; n=48) were used in a 2 x 2 x 2 x 2 factorial experiment. Treatments included two ages at slaughter, 12 to 13 month of age, termed the calf-fed steers, and 18 to 20 month of age, the yearling-fed steers. Hormonal growth implants were also administered (IMP) or not (NOIMP), and ractopamine hydrochloride was supplemented (RAC) or not (NORAC). A complete description of steers and experimental treatments is described in Section 2.2.1. Steer slaughter details are described in Section 2.2.2.

4.2.2 Steak preparation and assessment of the Warner-Bratzler shear force

Muscles analyzed for shear force (SF) were the m. *semitendinosus* (ST; eye of round) and m. *gluteus medius* (GM; top sirloin). Maximum peak SF was measured after an ageing period of seven days. A complete description of cooking protocol is described in Section 3.2.3. Warner-Bratzler shear force (WBSF) measurement protocol is described in Section 3.2.5.

Starting point for each SF curve was designated to be when the curve reached 0.5 kg of force, as visually that was the point where the blade started to compress the core and where the SF slope started to increase rapidly. The end point of each SF curve was set at 33 mm and was calculated by averaging the distance at which SF declined to 0.5 kg. Two macros were built (Texture Exponent 32 Software, TextureTechnologies Corp., Hamilton, MA) to estimate the myofibrillar (MF-SF) and connective tissue (CT-SF) shear forces. MF-SF was designated as the peak force occurring in the first half of the SF graph and CT-SF was the peak force occurring from 24 to 33 mm after the blade started shearing through the core (Pietrasik et al., 2009). Both MF-SF and CT-SF were measured on each SF graph generated by each of six cores from the GM and ST muscles from each animal. MF-SF and CT-SF values for each of the six cores were averaged for each muscle within each steer.

4.2.3 Statistical analysis

CT-SF and MF-SF data were analyzed as a 2 x 2 x 2 x 2 factorial design using PROC MIXED (SAS Version 9.2, SAS institute Inc., Cary, NC) with sources of variation including age at slaughter, implantation strategy, ractopamine hydrochloride feed supplementation, breed cross, and their two-, three-, and four-way interactions. Pen was included as a random effect. Denominator degrees of freedom were calculated using the Kenward-Roger approximation. For significant main or interaction effects ($P < 0.05$), differences between treatment or interaction means were computed using least square means and separated using *t*-tests with the PDIFF option.

Pearson correlations (PROC CORR; SAS Version 9.2, SAS institute Inc., Cary, NC) were used to test for linear relationships between maximum MF-SF, CT-SF, and peak SF and the inherent properties and meat quality characteristics of meat measured as described in Chapters 2 and 3, respectively. Twenty-six comparisons were made per SF, so a Bonferroni correction was used to correct the α significance value (26 comparisons for each SF, $\alpha = (0.05/26)$) and the correlations were significant at $P < 0.002$.

Data from the ST and GM were analyzed using multiple regression techniques to partition factors affecting maximum peak SF, MF-SF, and CT-SF by muscle. Inclusion of variables into the model was based on the influence of the variable on the SF test as determined using a simple linear regression analysis (PROC REG; SAS Version 9.2, SAS institute Inc., Cary, NC). Variables entered in the model were determined not to be correlated to each other, with correlation indicated by significance at $P < 0.05$. Multiple regression analyses on maximum peak SF, CT-SF, and MF-SF with the selected variables were performed using PROC REG (SAS Version 9.2, SAS institute Inc., Cary, NC) with the stepwise selection function.

4.3 RESULTS

4.3.1 Treatment effects on myofibrillar and connective tissue shear forces

Results for the treatment differences are presented in Table 4-1 and Figure 4-2. Statistical analysis revealed that age at slaughter had a significant effect on MF-SF of

cooked ST steaks, with mean MF-SF of yearling-fed steer ST steaks being greater ($P = 0.01$) than that of the calf-fed steer ST steaks. In the GM, only breed crosses yielded significantly different mean MF-SF values, with cooked GM steaks from the CRA steer carcasses having a greater ($P = 0.038$) mean MF-SF value than those from the HAA steer carcasses. There was a trend for greater ($P = 0.076$) mean MF-SF value in cooked GM steaks when from yearling-fed steers than from calf-fed steers.

Cooked ST steaks yielded greater ($P < 0.0001$) mean CT-SF value when from the yearling-fed steer carcasses than when from calf-fed steer carcasses, and when from implanted ($P = 0.005$) than non-implanted steer carcasses. The interaction between age at slaughter and breed cross indicated that cooked GM steaks from the CRA steer carcasses had a greater ($P = 0.003$) mean CT-SF value than those from the HAA steer carcasses when steers were calf-fed finished, but not when steers were yearling-fed finished (Figure 4-2).

4.3.2 Correlation analysis

The correlation coefficients for maximum MF-SF, CT-SF, and peak SF are presented in Table 4-2 for both the ST and GM muscles. Fast oxidative glycolytic (FOG) ($P < 0.0001$) and fast glycolytic (FG) ($P < 0.0001$) fibre cross-sectional areas were positively correlated with MF-SF. Conversely, ST MF-SF was not correlated with fibre frequency, sarcomere length, and total and soluble collagen contents. The following variables were also correlated with MF-SF in the ST: muscle weight ($P < 0.0001$), lightness (L*) ($P = 0.0001$), cooking loss ($P < 0.0001$), and purge loss ($P = 0.0001$). Correlation of CT-SF with soluble collagen content was significant ($P = 0.001$) for the ST as well as with FOG ($P = 0.001$) and FG ($P = 0.0002$) fibre distribution, and slow oxidative (SO) ($P = 0.0003$), FOG ($P = 0.0002$), and FG ($P = 0.0003$) cross-sectional areas. For the ST, other variables correlated with CT-SF were muscle weight ($P < 0.0001$), L* ($P < 0.0001$), chroma ($P = 0.0003$), hue ($P < 0.0001$), metmyoglobin (MMB) relative content ($P < 0.0001$), cooking time ($P < 0.0001$), standard deviation of shear force (STD) ($P < 0.0001$), and purge loss ($P = 0.002$). All the fibre cross-sectional areas ($P < 0.0001$) and FG% ($P = 0.001$) were correlated with maximum ST peak SF. Muscle weight was also correlated ($P < 0.0001$) with maximum peak SF in the ST as well

as L* ($P < 0.0001$), hue ($P < 0.0001$), STD ($P = 0.0003$), and purge loss ($P = 0.0001$). Total and soluble collagen contents were not correlated with maximum peak ST SF.

In the GM, MF-SF was positively correlated with FOG ($P = 0.0002$) and FG ($P = 0.001$) fibre cross-sectional areas. MF-SF was not correlated with total and soluble collagen contents in the GM. Other variables correlated with MF-SF in the GM were muscle weight ($P < 0.0001$), chroma ($P < 0.0001$), cooking loss ($P < 0.0001$), cooking time ($P = 0.0001$), and deoxymyoglobin (DMB) ($P < 0.0001$), MMB ($P = 0.0008$), and oxymyoglobin (OMB) ($P = 0.002$) relative contents. Soluble collagen content and CT-SF were also significantly correlated in the GM ($P < 0.0001$). Fibre characteristics correlated with CT-SF of the GM were SO ($P < 0.0001$), FOG ($P < 0.0001$), and FG ($P = 0.0001$) cross-sectional areas and SO% ($P = 0.002$). Other variables correlated with CT-SF in the GM were muscle weight ($P < 0.0001$), pH 24 hours (pH_{24h}) ($P = 0.0001$), chroma ($P = 0.0005$), MMB relative content ($P < 0.0001$), and STD ($P < 0.0001$). Maximum peak SF in the GM was correlated with SO ($P < 0.0001$), FOG ($P < 0.0001$), and FG ($P = 0.0003$) fibre cross-sectional areas. Soluble collagen content ($P = 0.006$) and muscle weight ($P < 0.0001$) were correlated with maximum peak SF in the GM. Total collagen content and maximum peak SF were not correlated. In the GM, pH_{24h} ($P = 0.002$), chroma ($P < 0.0001$), MMB ($P < 0.0001$) and DMB ($P < 0.0001$) relative contents, cooking loss ($P < 0.0001$), and STD ($P < 0.0001$) were significantly correlated with maximum peak SF.

4.3.3 Multiple regression

Simple linear regression significances between maximum peak SF, MF-SF, CT-SF, and muscle and meat characteristics are presented in Table 4-3. Correlation coefficients between muscle and meat characteristics are presented in Tables 4-4 and 4-5 for the ST and GM, respectively. Variables that were selected to be in the multiple regression analyses for the ST maximum peak SF and CT-SF were muscle weight, FG%, total collagen content, OMB relative content, moisture content, sarcomere length, and pH_{24h}. The multiple regression analysis of ST MF-SF was performed with muscle weight, cooking loss, FG%, sarcomere length, OMB relative content, pH_{24h}, and temperature 24 hours (T_{24h}). In the GM, multiple regressions for maximum peak SF and MF-SF were analyzed with muscle weight, cooking loss, FG%, pH_{24h}, pH 7 days (pH_{7d}), and T_{24h}. In

the analysis for GM CT-SF, variables selected for the analysis were muscle weight, FG%, cooking loss, pH_{24h}, and pH_{7d}.

Table 4-6 shows a summary of the regression analysis for the ST. The stepwise selection chose muscle weight ($P < 0.0001$), FG% ($P = 0.004$), moisture content ($P = 0.03$), and OMB relative content ($P = 0.15$) to be in the regression equation for maximum peak SF in the ST. The multiple regression analysis for maximum MF-SF of the ST selected muscle weight ($P < 0.0001$), cooking loss ($P < 0.0001$), and pH_{24h} ($P = 0.08$). The CT-SF regression equation included muscle weight ($P < 0.0001$), FG% ($P = 0.0005$), moisture content ($P = 0.01$), total collagen content ($P = 0.06$), and OMB relative content ($P = 0.13$). The SF regression equation that explained the most variation in SF was the MF-SF equation with 46.0% of the variation explained compared to 45.3% for the CT-SF regression equation and 43.7% for the maximum peak SF regression equation.

Regression equations for the ST SF were:

$$\text{Peak SF} = 27.54247 + 0.00192*(\text{muscle weight}) + 0.03876*(\text{FG\%}) - 0.33124*(\text{moisture content}) - 3.15732*(\text{OMB relative content})$$

$$\text{MF-SF} = -7.98145 + 0.00134*(\text{muscle weight}) + 0.01111*(\text{cooking loss}) + 1.51749*(pH_{24h})$$

$$\text{CT-SF} = 32.18296 + 0.00192*(\text{muscle weight}) + 0.05124*(\text{FG\%}) - 0.42263*(\text{moisture content}) + 0.05564*(\text{total collagen content}) - 3.59338*(\text{OMB relative content})$$

Summary results for the regressions in the GM are presented in Table 4-7. For the GM, the multiple regression analysis kept cooking loss ($P < 0.0001$), muscle weight ($P < 0.0001$), pH_{24h} ($P = 0.007$), and pH_{7d} ($P = 0.02$) to represent maximum peak SF. GM MF-SF was best represented by cooking loss ($P < 0.0001$), muscle weight ($P = 0.0002$), pH_{7d} ($P = 0.06$), and pH_{24h} ($P = 0.1$). The stepwise selection chose muscle weight ($P < 0.0001$), pH_{24h} ($P = 0.0004$), cooking loss ($P = 0.02$), and FG% ($P = 0.06$) in the regression equation of GM CT-SF. The MF-SF regression equation was the one

explaining most of the variation in SF with 54.4% compared to 52.2% for the maximum peak SF regression equation and 41.7% for the CT-SF regression equation.

Regression equations for the GM were:

$$\text{Peak SF} = 21.60412 + 0.01502*(\text{cooking loss}) + 0.00064126*(\text{muscle weight}) - 2.22858*(pH_{24h}) - 1.61196*(pH_{7d})$$

$$\text{MF-SF} = 13.93479 + 0.02056*(\text{cooking loss}) + 0.00044072*(\text{muscle weight}) - 1.37250*(pH_{7d}) - 1.23776*(pH_{24h})$$

$$\text{CT-SF} = 16.65015 + 0.00067478*(\text{muscle weight}) - 2.56857*(pH_{24h}) + 0.00454*(\text{cooking loss}) - 0.0207*(FG\%)$$

4.4 DISCUSSION

4.4.1 Treatment effects on myofibrillar and connective tissue shear forces

MF-SF and CT-SF values were affected differently by the various production treatments and appeared representative of the myofibrillar and connective tissue contributions to SF. In the GM, the increase in collagen content and the decrease of its solubility that occurred with steer age (Section 2.3.4) mirrored the increase in CT-SF with age at slaughter. The increase in CT-SF with age was not surprising given that soluble collagen content is known to decrease with cattle age (Hill, 1966) and that heat insoluble cross-links forming in maturing collagen contribute to meat toughness (Shimokomaki et al., 1972). The increase in CT-SF with age at slaughter was particularly marked in the muscles from the HAA crossbred steer carcasses, suggesting that their collagen matured very quickly in the time that elapsed between calf-fed and yearling-fed finishing times. This is probably related to the time at which cattle reach physiological maturity because British breeds reach puberty earlier in their lifetime than Continental breeds (Gregory et al., 1999). So, collagen may form mature cross-links earlier in the lifetime of British cattle than Continental cattle. This interaction between breed crosses and age at slaughter was however not observed in the present study. In yearling-fed steers, the soluble collagen fraction tended to decrease with age (Section 2.3.4) while CT-SF increased.

These observations suggested that the soluble collagen is responsible for the changes in ST CT-SF. Nevertheless, ST CT-SF increased with IMP without observable changes in collagen solubility, but increases in muscle fibre cross-sectional areas were observed. In this case, it appeared that CT-SF rather than MF-SF detected the increase in SF caused by the muscle fibres. Why this occurred was unclear, as IMP has been shown to increase SF by increasing the protease inhibitor activity, calpastatin and cystatin, rather than decreasing soluble collagen content (Gerken et al., 1995).

MF-SF of the GM tended to increase in yearling-fed steers in association with SO and FOG cross-sectional areas increases (Section 2.3.3). Similarly, for the ST, the increase in the cross-sectional areas of SO and FOG fibres was accompanied by an increase in MF-SF. Increases in GM MF-SF from carcasses of CRA crossbred steers were also associated with larger FG fibre cross-sectional areas than those of the HAA steer carcasses. So, increases in fibre cross-sectional areas caused increases in MF-SF in the current study, result that were similar to other studies (Crouse et al., 1991; Herring et al., 1965; Seideman et al., 1987) where increased muscle fibre size was associated with increased SF value as well. Reason for the why increasing fibre cross-sectional areas would increase MF-SF was not investigated in these studies.

Overall, age at slaughter accounted for most of the variation observed in CT-SF and MF-SF and suggested that changes in soluble collagen content and the muscle fibre cross-sectional area contributed most to increases in overall peak SF. Except for the IMP treatment in the ST, all variations in MF-SF and CT-SF occurred with changes in fibre cross-sectional areas and/or soluble collagen content. Increases in peak SF with hormonal implants have been attributed to increased protease inhibitor levels, calpastatin and cystatin, rather than decreased soluble collagen content (Gerken et al., 1995).

4.4.2 Correlation analysis

MF-SF, CT-SF, and peak SF were all correlated to some extent with myofibre distribution and/or cross-sectional areas. Thus, MF-SF did not clearly differentiate the myofibrillar component of SF as expected, although fibre cross-sectional area correlation coefficients in the ST were slightly stronger with MF-SF than with CT-SF and maximum peak SF. The opposite was observed in the GM. Correlation coefficients between fibre

cross-sectional areas and SF were all significant and positive, meaning that as muscle fibres enlarged, SF increased. Herring et al. (1965) also found that as the muscle fibre diameter increased, tenderness decreased. Muscle fibre cross-sectional areas were more indicative of SF than fibre distribution. Nevertheless, that SF was not much affected by muscle fibre distribution was partially unexpected because other research showed that the fibre distribution was correlated to SF (Crouse et al., 1991; Lewis Jr et al., 1977; Seideman et al., 1987), sometimes more than the fibre cross-sectional areas were (Seideman et al., 1986). As for sarcomere length, it has been observed to affect SF of the m. *longissimus* (Koohmaraie et al., 1996; Steen et al., 1997) although not always (Fiems et al., 1990; Hall and Hunt, 1982). The impact of sarcomere shortening on the m. *longissimus* may not be the same on the ST and GM muscles, but in the current experiment, either the extent of sarcomere shortening post mortem did not affect MF-SF or the contribution of sarcomeres to SF was not reflected by peak MF-SF value.

Correlation coefficients between soluble collagen content and CT-SF of both muscles were stronger than that of soluble collagen and maximum peak SF, and MF-SF was not correlated at all with soluble collagen content. These results indicated that CT-SF represented the connective tissue component of SF better than MF-SF and maximum peak SF did. Also, CT-SF was negatively correlated with soluble collagen content but not correlated with total collagen content, reinforcing the hypothesis that soluble collagen content is a better indicator of SF than total collagen content. This was expected because during cooking, the insoluble collagen fraction, with its trivalent heat insoluble cross-links, is the one contributing to SF the most (Lepetit, 2008; Shimokomaki et al., 1972).

Crouse et al. (1991) and Seideman et al. (1987) observed positive correlations between fibre cross-sectional areas and maximum peak SF, although correlations in the former study were not significant after three days of ageing most likely because of the extent of proteolysis on muscle fibres during ageing. Moreover, Whipple et al. (1990) observed that at one, three, seven, and fourteen days post mortem, myofibrillar fragmentation index, which is indicative of the proteolysis of muscle fibres, increased as WBSF decreased. In the current experiment, ageing lasted seven days, which allowed time for proteolysis to occur. Weakening of connective tissue occurs later during ageing of the meat, starting at about nine to ten days post mortem (Nishimura et al., 1998; Pambuka et al., 2007). So, the time at which myofibrillar and connective tissue

proteolysis occurs may account for why the myofibrillar component, and not the connective tissue component, was similar across maximum peak SF, MF-SF, and CT-SF. Therefore, CT-SF represented the connective tissue component of SF better than MF-SF represented the myofibrillar component of SF.

4.4.3 Multiple regression

Determining a single regression equation for both ST and GM was not possible because variables contributing to SF selected for inclusion in the multiple regression models were different between muscles. Some variables however were present in the regression equations of both muscles and these were: muscle weight for maximum peak SF and CT-SF, and muscle weight, cooking loss, and pH_{24h} for MF-SF. Also, regression equations were similar across maximum peak SF, CT-SF, and MF-SF of each muscle because muscle weight was present in all the regression equations of the ST, and muscle weight, cooking loss, and pH_{24h} were present in all the regression equations of the GM.

Muscle weight was the variable that accounted for most of the variation in maximum peak SF, CT-SF, and MF-SF for the ST. Muscle weight was also positively correlated with all the fibre cross-sectional areas and negatively correlated with soluble collagen content. This means that as muscle weight increased, fibre cross-sectional areas increased and collagen solubility decreased, resulting in tough meat. Increases in muscle weight and fibre cross-sectional areas, and a trend towards decreased collagen solubility were all observed (Sections 2.3.1, 2.3.3, and 2.3.4, respectively) in ST from yearling-fed steer carcasses compared to ST from calf-fed steer carcasses. In the GM, muscle weight explained variations in CT-SF only. Changes occurring with age in the GM also involved increased muscle weight with increased total collagen content and decreased collagen solubility (Sections 2.3.1 and 2.3.4). Similar results were observed with age and growth of steers on muscle fibre cross-sectional areas (Jurie et al., 1995; Wegner et al., 2000) and collagen solubility (Gerrard et al., 1987). So, it is most likely that muscle weight reflected changes in muscle fibres and collagen solubility on SF that occurred with biological maturity of the steers. Muscle weight was an indicator of meat SF in this case but may not be in steers older than 20 months. Once maturity is reached and growth stops, muscle weight stabilizes and remains the same, but collagen solubility may still decrease (Hill, 1966).

In the ST, the SF regression equation that explained most of the variation in SF was the MF-SF equation and this equation was the only one analyzed for the multiple regression analysis with cooking loss. Indeed, this variable was not included in maximum peak SF and CT-SF models because these SF values were more correlated with FG% than with cooking loss. Also, cooking loss was excluded from those equations because it was correlated with FG%. In the GM, the MF-SF regression equation was also the one explaining most of the variation in SF and cooking loss explained 45% of its variation. Cooking loss was positively correlated with MF-SF in both muscles, meaning that as water losses increased with cooking, SF increased as well. During cooking, water losses are due to water evaporation (Aberle et al., 2001), protein denaturation (Cheng and Sun, 2008), collagen fibre shrinkage (Rao et al., 1989), and sarcomere shortening (Bouton et al., 1975a). Cooking loss was also selected to be in the regression equation of GM CT-SF. Collagen fibres shrink during cooking (Lepetit, 2008) and contribute to increased meat toughness (Bouton et al., 1981). The extent of collagen fibre shrinkage may not have been that important in the current study because cooking loss explained only 3.2% of the variation in GM CT-SF. Therefore, cooking loss indicated changes occurring to muscle fibres and proteins, and their hydration state more than changes occurring to collagen fibres, as supported by Beilken et al. (1986) and Bouton et al. (1976). In the experiment of Seideman et al. (1987), although the fibre cross-sectional areas were correlated with maximum peak SF, they were also not selected by the partial regression analysis to represent SF. Myofibrillar index, indicative of the degree of proteolysis of muscle fibres post mortem, was selected instead. So, measurements providing information on the muscle fibre and protein states appeared to be better indicators of SF than the type of muscle fibres themselves.

The disparity between MF-SF regression equations of the GM and ST suggested that the myofibrillar component did not behave the same way in both muscles and was more important in the GM than the ST, as observed by Bouton et al. (1975b). Indeed, muscle weight was correlated with several variables in both muscles and was the variable explaining most of the variation in ST MF-SF. Nevertheless, in the GM, cooking loss explained most of the variation in MF-SF and, as explained previously, it was probably indicative of the muscle fibre and protein states. Another observation that suggests the myofibrillar component is more implicated in SF of the GM than the ST is the selection of pH in the regression equation of ST maximum peak SF and CT-SF. The muscle pH_{24h}

was negatively correlated with these SF measures, meaning that as this pH decreased, SF increased. Silva et al. (1999) showed that beef with normal ultimate pH values of 5.7 was tougher than beef with ultimate pH values of 6.1 to 6.5 and this was explained by the greater proteolytic enzyme activity at high pH values than normal values. Indeed, the proteolytic calpain enzymes have optimal activities between pH values of 6.5 to 8.0 (Etherington, 1984). Inclusion of pH_{24h} in the regression equations of the GM and not in that of the ST suggested that GM SF relied more on post mortem muscle fibre proteolysis within 24 hours than ST SF.

Maximum peak SF and CT-SF regression equations for the ST were almost the same because the multiple regression analyses were performed with the same variables except that CT-SF included total collagen content in its equation. Although total collagen content explained only 1.88% of the variation in ST CT-SF, this indicated that CT-SF really detected a portion of SF caused by the connective tissue. It would have been interesting to evaluate the contribution of soluble collagen content to CT-SF because it should be the characteristic contributing the most to increase CT-SF (Shimokomaki et al., 1972). Soluble collagen content was however correlated with muscle weight and could not be included in the regression analysis. In the GM, muscle weight was correlated with both total and soluble collagen contents, so none of these variables could be included in the CT-SF model. Maximum peak SF and MF-SF of the GM had almost the same regression equations, again most likely because their multiple regression analyses were performed with the same set of variables. It is interesting to note that regression equations were almost the same between maximum GM peak SF and MF-SF, and ST peak SF and CT-SF. Bouton et al. (1975b) observed that initial SF yield, representing the myofibrillar structure, was greater in the GM than the ST. Also, the greater SF in the ST than in the GM is known (Herring et al., 1965) and pertains to the high collagen content of the ST (Bendall, 1967). This is another indication that GM SF relies more on muscle fibre characteristics than the ST SF, and that the ST SF relies more on the connective tissue characteristics than the GM SF.

4.5 CONCLUSION

Determining a single regression equation to predict SF was not possible because equations most likely are different for every single muscle, which in turns makes SF

difficult to predict. Similarities may be observed among equations but as muscles have different fibre patterns and collagen contents, a SF regression equation for a specific muscle may not be relevant to another muscle. Age at slaughter was the factor affecting SF the most. As steers age and grow, their muscle fibres enlarge and collagen solubility decreases, causing an overall increase in SF. This relationship may however not be true when steers are older than 20 months because muscle growth and collagen solubility may not vary the same way after that age. GM SF was more related to the myofibrillar than to the connective tissue component of meat whereas the opposite was observed for ST SF. Cooking loss was a good indicator of the myofibrillar component, but the connective tissue component was not related to any specific variable other than muscle weight.

Table 4-1 Effect of age at slaughter, implantation strategy, ractopamine hydrochloride feed supplementation, and breed cross on myofibrillar (MF-SF) and connective tissue shear force (CT-SF) of the m. *semitendinosus* (ST) and m. *gluteus medius* (GM)

Variable	Age at slaughter		Implantation		Ractopamine feeding		SEM[5]	Breed cross		SEM
	Calf-fed	Yearling-fed	NOIMP[1]	IMP[2]	NORAC[3]	RAC[4]		HAA[6]	CRA[7]	
n=	56	56	56	56	56	56	-	64	48	-
ST										
MF-SF (kg)	6.18[b]	7.40[a]	6.46	7.13	6.59	7.00	0.25	6.70	6.89	0.21
CT-SF (kg)	5.34[b]	7.72[a]	6.01[b]	7.05[a]	6.53	6.52	0.19	6.57	6.49	0.16
GM										
MF-SF (kg)	5.14	5.98	5.37	5.74	5.43	5.69	0.29	5.34[b]	5.77[a]	0.23
CT-SF[8] (kg)	4.27[b]	5.54[a]	4.68	5.13	4.95	4.87	0.17	4.83	4.98	0.14

[1] No hormonal implant

[2] Hormonal implants

[3] No ractopamine hydrochloride feed supplementation

[4] Ractopamine hydrochloride feed supplementation

[5] Pooled standard error of the mean (SEM) for age at slaughter, implantation strategy, and ractopamine feed supplementation

[6] Crossbred Hereford-Aberdeen Angus

[7] Crossbred Charolais-Red Angus

[8] An interaction involving age at slaughter and breed cross was significant (Figure 4-2)

[ab] Means within the same row within a main effect with different superscript letters are significantly different ($P < 0.05$)

Table 4-2 Pearson correlation coefficients between myofibrillar (MF-SF), connective tissue (CT-SF), peak shear force (SF), and muscle and meat characteristics of the m. *gluteus medius* (GM) and m. *semitendinosus* (ST)

Variable	MF-SF		CT-SF		Peak SF	
	GM	ST	GM	ST	GM	ST
SO%[1,2]	0.19	-0.07	0.30*	-0.14	0.25	-0.16
FOG%[3]	0.00	-0.17	-0.04	-0.30*	-0.07	-0.23
FG%[4]	-0.15	0.19	-0.20	0.35*	-0.15	0.30*
SO area[5]	0.29	0.47**	0.52**	0.34*	0.39**	0.42**
FOG area	0.35*	0.42**	0.48**	0.35*	0.42**	0.41**
FG area	0.30*	0.42**	0.36*	0.33*	0.34*	0.40**
Muscle weight	0.37**	0.56**	0.54**	0.56**	0.48**	0.59**
Total collagen	0.09	0.03	0.21	0.22	0.13	0.13
Soluble collagen[6]	-0.25	-0.21	-0.39**	-0.31*	-0.32*	-0.29
Fat content	-0.12	0.01	-0.17	-0.01	-0.18	0.00
Protein content	0.03	0.08	-0.12	0.16	-0.01	0.10
Moisture content	0.17	-0.07	0.26	-0.14	0.25	-0.10
pH_{24h}	-0.20	0.07	-0.36*	0.02	-0.30*	0.05
T_{24h}	-0.10	0.07	-0.11	0.10	-0.15	0.09
pH_{7d}	-0.21	-0.16	-0.12	0.08	-0.22	-0.05
Sarcomere length	-0.02	-0.13	-0.04	0.00	-0.01	-0.07
Lightness	0.07	-0.35*	-0.13	-0.48**	0.02	-0.42**
Chroma	0.40**	0.12	0.32*	0.33*	0.40**	0.26
Hue	0.04	-0.26	-0.22	-0.45**	-0.03	-0.37**
MMB[7]	0.31*	0.20	0.44**	0.36**	0.39**	0.28
DMB[8]	-0.38**	0.01	-0.28	-0.01	-0.38**	0.00
OMB[9]	0.29*	-0.09	0.10	-0.14	0.25	-0.12
Purge loss	0.12	0.36*	0.08	0.30*	0.11	0.36*
Cooking loss	0.67**	0.44**	0.29	-0.24	0.55**	0.14
Cooking time	0.36*	0.00	0.02	-0.49**	0.23	-0.22
STD[10]	0.22	0.08	0.42**	0.40**	0.38**	0.34*

[1] Fibre distribution
[2] Slow oxidative muscle fibre
[3] Fast oxidative glycolytic muscle fibre
[4] Fast glycolytic muscle fibre
[5] Fibre cross-sectional areas
[6] Expressed as a percentage of total collagen content
[7] Metmyoglobin relative content
[8] Deoxymyoglobin relative content
[9] Oxymyoglobin relative content
[10] Standard deviation of shear force
* $P < 0.002$
** $P < 0.0001$

Table 4-3 Regression P-values between myofibrillar (MF-SF), connective tissue (CT-SF), peak shear force (SF), and muscle and meat characteristics of the m. *gluteus medius* (GM) and m. *semitendinosus* (ST)

Variable	MF-SF		CT-SF		Peak SF	
	GM	ST	GM	ST	GM	ST
SO%[1,2]	0.05	0.45	0.002	0.15	0.008	0.08
FOG%[3]	0.98	0.08	0.69	0.001	0.47	0.01
FG%[4]	0.13	0.047	0.03	0.0002	0.13	0.001
SO area[5]	0.002	<.0001	<.0001	0.0003	<.0001	<.0001
FOG area	0.0002	<.0001	<.0001	0.0002	<.0001	<.0001
FG area	0.001	<.0001	0.0001	0.0003	0.0003	<.0001
Muscle weight	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Total collagen	0.33	0.76	0.02	0.02	0.17	0.16
Soluble collagen[6]	0.008	0.02	<.0001	0.001	0.0006	0.002
Fat content	0.20	0.93	0.08	0.95	0.05	0.97
Protein content	0.76	0.37	0.22	0.10	0.96	0.30
Moisture content	0.07	0.43	0.006	0.13	0.007	0.29
pH_{24h}	0.03	0.44	0.0001	0.84	0.002	0.62
T_{24h}	0.31	0.48	0.23	0.29	0.13	0.36
pH_{7d}	0.02	0.10	0.20	0.42	0.02	0.58
Sarcomere length	0.84	0.18	0.70	0.98	0.92	0.49
Lightness	0.48	0.0001	0.16	<.0001	0.87	<.0001
Chroma	<.0001	0.22	0.0005	0.0003	<.0001	0.006
Hue	0.70	0.005	0.02	<.0001	0.75	<.0001
MMB[7]	0.0008	0.04	<.0001	<.0001	<.0001	0.002
DMB[8]	<.0001	0.93	0.003	0.89	<.0001	0.10
OMB[9]	0.002	0.34	0.28	0.15	0.008	0.20
Purge loss	0.20	0.0001	0.41	0.002	0.23	0.0001
Cooking loss	<.0001	<.0001	0.002	0.02	<.0001	0.14
Cooking time	0.0001	0.99	0.82	<.0001	0.01	0.02
STD[10]	0.02	0.41	<.0001	<.0001	<.0001	0.0003

[1] Fibre distribution
[2] Slow oxidative muscle fibre
[3] Fast oxidative glycolytic muscle fibre
[4] Fast glycolytic muscle fibre
[5] Fibre cross-sectional area
[6] Expressed as a percentage of total collagen content
[7] Metmyoglobin relative content
[8] Deoxymyoglobin relative content
[9] Oxymyoglobin relative content
[10] Standard deviation of shear force
* $P < 0.002$
** $P < 0.0001$

Table 4-4a Pearson correlation coefficients between muscle and meat characteristics of the m. *semitendinosus*

Variable	SO%[1,2]	FOG%[3]	FG%[4]	SO area[5]	FOG area	FG area	Muscle weight	Total collagen	Soluble collagen[6]	Fat content	Protein content	Moisture content
SO%	1.00	-0.11	-0.47*	0.10	0.09	0.06	0.03	-0.10	0.22*	-0.17	-0.05	0.23*
FOG%	-0.11	1.00	-0.83*	-0.01	0.11	0.19	-0.18	-0.03	0.05	0.09	-0.13	0.01
FG%	-0.47*	-0.83*	1.00	-0.05	-0.14	-0.20*	0.14	0.08	-0.17	0.02	0.14	-0.14
SO area	0.10	-0.01	-0.05	1.00	0.70*	0.61*	0.50*	0.08	-0.20*	0.02	0.11	-0.13
FOG area	0.09	0.11	-0.14	0.70*	1.00	0.76*	0.58*	0.04	-0.08	-0.10	0.07	0.03
FG area	0.06	0.19*	-0.20*	0.61*	0.76*	1.00	0.58*	0.08	-0.20*	-0.08	0.13	-0.01
Muscle weight	0.03	-0.18	0.14	0.50*	0.58*	0.58*	1.00	0.11	-0.27*	-0.31*	0.25*	0.16
Total collagen	-0.10	-0.03	0.08	0.08	0.04	0.08	0.11	1.00	-0.60*	0.02	-0.07	0.00
Soluble collagen	0.22*	0.05	-0.17	-0.20*	-0.08	-0.20*	-0.27*	-0.60*	1.00	-0.01	-0.16	0.11
Fat content	-0.17	0.09	0.02	0.02	-0.10	-0.08	-0.31*	0.02	-0.01	1.00	-0.56*	-0.86*
Protein content	-0.05	-0.13	0.14	0.11	0.07	0.13	0.25*	-0.07	-0.16	-0.56*	1.00	0.09
Moisture content	0.23*	0.01	-0.14	-0.13	0.03	-0.01	0.16	0.00	0.11	-0.86*	0.09	1.00
pH_{24h}	0.08	-0.01	-0.04	0.01	0.15	0.13	0.03	-0.01	0.05	0.09	-0.06	-0.06
T_{24h}[7]	-0.06	0.06	-0.02	0.01	0.02	0.14	0.15	0.20*	-0.26*	0.01	0.07	-0.08
pH_{7d}	0.09	-0.36*	0.27*	-0.06	-0.06	-0.18	-0.02	-0.04	0.08	0.02	-0.19*	0.03
Sarcomere length	-0.27*	0.08	0.08	-0.10	-0.14	-0.08	-0.10	-0.10	-0.03	0.11	0.00	-0.13
Lightness	0.00	0.26*	-0.24*	-0.34*	-0.31*	-0.35*	-0.44*	-0.17	0.15	0.11	-0.29*	0.08
Chroma	0.06	-0.19*	0.14	0.19*	0.15	0.03	0.21*	0.12	-0.08	-0.17	0.10	0.11
Hue	-0.05	0.34*	-0.28*	-0.28*	-0.25*	-0.15	-0.24*	-0.11	0.05	0.02	-0.08	0.07
MMB[8]	0.10	-0.19	0.11	0.33*	0.22*	0.17	0.36*	0.32*	-0.30*	-0.09	0.05	0.04
DMB[9]	0.02	-0.08	0.06	0.05	0.06	-0.03	-0.15	-0.05	0.14	0.07	-0.18	0.01
OMB[10]	-0.06	0.16	-0.11	-0.19*	-0.15	-0.04	0.00	-0.08	-0.02	-0.04	0.16	-0.03

Purge loss	-0.04	-0.04	0.06	0.23*	0.10	0.30*	0.43*	0.21*	-0.31*	-0.28*	0.16	0.24*
Cooking loss	0.16	0.13	-0.20*	0.18	0.13	0.18	0.13	-0.14	0.06	-0.08	-0.17	0.21*
Cooking time	0.08	0.19	-0.22*	-0.09	-0.20*	-0.19	-0.40*	-0.12	0.15	0.08	-0.25*	0.09
STD[11]	-0.26*	-0.13	0.27*	0.00	0.13	0.08	0.24*	0.06	-0.11	-0.16	0.11	0.11

[1] Fibre distribution

[2] Slow oxidative muscle fibre

[3] Fast oxidative glycolytic muscle fibre

[4] Fast glycolytic muscle fibre

[5] Fibre cross-sectional areas

[6] Expressed as a percentage of total collagen content

[7] Temperature at 24 hours post mortem

[8] Metmyoglobin relative content

[9] Deoxymyoglobin relative content

[10] Oxymyoglobin relative content

[11] Standard deviation of shear force

*P < 0.05

Table 4-4b Pearson correlation coefficients between muscle and meat characteristics of the m. *semitendinosus*

Variable	pH_{24h}	T_{24h}[1]	pH_{7d}	Sarcomere length	Lightness	Chroma	Hue	MMB[2]	DMB[3]	OMB[4]	Purge loss	Cooking loss	Cooking time	STD[5]
SO%[6,7]	0.08	-0.06	0.09	-0.27*	0.00	0.06	-0.05	0.10	0.02	-0.06	-0.04	0.16	0.08	-0.26*
FOG%[8]	-0.01	0.06	-0.36*	0.08	0.26*	-0.19*	0.34*	-0.19	-0.08	0.16	-0.04	0.13	0.19	-0.13
FG%[9]	-0.04	-0.02	0.27*	0.08	-0.24*	0.14	-0.28*	0.11	0.06	-0.11	0.06	-0.20*	-0.22*	0.27*
SO area[10]	0.01	0.01	-0.06	-0.10	-0.34*	0.19*	-0.28*	0.33*	0.05	-0.19*	0.23*	0.18	-0.09	0.00
FOG area	0.15	0.02	-0.06	-0.14	-0.31*	0.15	-0.25*	0.22*	0.06	-0.15	0.10	0.13	-0.20	0.13
FG area	0.13	0.14	-0.18	-0.08	-0.35*	0.03	-0.15	0.17	-0.03	-0.04	0.30*	0.18	-0.19*	0.08
Muscle weight	0.03	0.15	-0.02	-0.10	-0.44*	0.21*	-0.24*	0.36*	-0.15	0.00	0.43*	0.13	-0.40*	0.24*
Total collagen	-0.01	0.20*	-0.04	-0.10	-0.17	0.12	-0.11	0.32*	-0.05	-0.08	0.21*	-0.14	-0.12	0.06
Soluble collagen[11]	0.05	-0.26*	0.08	-0.03	0.15	-0.08	0.05	-0.30*	0.14	-0.02	-0.31*	0.06	0.15	-0.11
Fat content	0.09	0.01	0.02	0.11	0.11	-0.17	0.02	-0.09	0.07	-0.04	-0.28*	-0.08	0.08	-0.16
Protein content	-0.06	0.07	-0.19*	0.00	-0.29*	0.10	-0.08	0.05	-0.18	0.16	0.16	-0.17	-0.25*	0.11
Moisture content	-0.06	-0.08	0.03	-0.13	0.08	0.11	0.07	0.04	0.01	-0.03	0.24*	0.21*	0.09	0.11
pH_{24h}	1.00	0.16	-0.26*	-0.05	-0.16	-0.22*	-0.06	-0.28*	0.15	-0.04	-0.31*	-0.05	-0.13	0.03
T_{24h}	0.16	1.00	-0.20*	-0.07	-0.12	-0.20*	0.04	0.00	-0.08	0.09	0.09	-0.03	-0.17	0.07
pH_{7d}	-0.26*	-0.20*	1.00	0.25*	0.03	0.27*	-0.32*	0.13	0.26*	-0.32*	-0.14	-0.01	0.02	0.11
Sarcomere length	-0.05	-0.07	0.25*	1.00	0.04	-0.10	-0.03	-0.09	0.11	-0.08	-0.11	-0.11	0.02	0.09
Lightness	-0.16	-0.12	0.03	0.04	1.00	-0.19*	0.63*	-0.33*	-0.13	0.27*	-0.06	0.11	0.27*	-0.18
Chroma	-0.22*	-0.20*	0.27*	-0.10	-0.19*	1.00	-0.54*	0.71*	-0.20*	-0.09	0.23*	-0.03	-0.01	0.12
Hue	-0.06	0.04	-0.32*	-0.03	0.63*	-0.54*	1.00	-0.37*	-0.51*	0.68*	-0.01	0.13	0.13	-0.15
MMB	-0.28*	0.00	0.13	-0.09	-0.33*	0.71*	-0.37*	1.00	-0.27*	-0.14	0.34*	0.00	-0.06	0.01
DMB	0.15	-0.08	0.26*	0.11	-0.13	-0.20*	-0.51*	-0.27*	1.00	-0.92*	-0.25*	-0.04	0.04	0.05
OMB	-0.04	0.09	-0.32*	-0.08	0.27*	-0.09	0.68*	-0.14	-0.92*	1.00	0.11	0.05	-0.02	-0.06

Purge loss	-0.31*	0.09	-0.14	-0.11	-0.06	0.23*	-0.01	0.34*	-0.25*	0.11	1.00	0.17	-0.09	0.09
Cooking loss	-0.05	-0.03	-0.01	-0.11	0.11	-0.03	0.13	0.00	-0.04	0.05	0.17	1.00	0.76*	-0.22*
Cooking time	-0.13	-0.17	0.02	0.02	0.27*	-0.01	0.13	-0.06	0.04	-0.02	-0.09	0.76	1.00	-0.31*
STD	0.03	0.07	0.11	0.09	-0.18	0.12	-0.15	0.01	0.05	-0.06	0.09	-0.22*	-0.31*	1.00

[1] Temperature at 24 hours post mortem

[2] Metmyoglobin relative content

[3] Deoxymyoglobin relative content

[4] Oxymyoglobin relative content

[5] Standard deviation of shear force

[6] Fibre distribution

[7] Slow oxidative muscle fibre

[8] Fast oxidative glycolytic muscle fibre

[9] Fast glycolytic muscle fibre

[10] Fibre cross-sectional area

[11] Expressed as a percentage of total collagen content

*$P < 0.05$

Table 4-5a Pearson correlation coefficients between muscle and meat characteristics of the m. *gluteus medius*

Variable	SO%[1,2]	FOG%[3]	FG%[4]	SO area[5]	FOG area	FG area	Muscle weight	Total collagen	Soluble collagen[6]	Fat content	Protein content	Moisture content
SO%	1.00	-0.15	-0.67*	0.38*	0.50*	0.31*	0.26*	0.14	-0.31*	-0.17	-0.11	0.23*
FOG%	-0.15	1.00	-0.63*	0.04	0.01	0.02	-0.12	-0.15	0.02	0.23*	-0.05	-0.22*
FG%	-0.67*	-0.63*	1.00	-0.33*	-0.40*	-0.26*	-0.12	0.00	0.23*	-0.04	0.13	-0.02
SO area	0.38*	0.04	-0.33*	1.00	0.83*	0.65*	0.46*	0.11	-0.31*	-0.09	-0.29*	0.28*
FOG area	0.50*	0.01	-0.40*	0.83*	1.00	0.75*	0.43*	0.12	-0.35*	-0.15	-0.25*	0.32*
FG area	0.31*	0.02	-0.26*	0.65*	0.75*	1.00	0.31*	0.12	-0.28*	-0.19*	-0.10	0.30*
Muscle weight	0.26*	-0.12	-0.12	0.46*	0.43*	0.31*	1.00	0.19*	-0.34*	-0.32*	-0.04	0.38*
Total collagen	0.14	-0.15	0.00	0.11	0.12	0.12	0.19*	1.00	-0.41*	-0.02	-0.12	0.09
Soluble collagen	-0.31*	0.02	0.23*	-0.31*	-0.35*	-0.28*	-0.34*	-0.41*	1.00	0.08	0.17	-0.16
Fat content	-0.17	0.23*	-0.04	-0.09	-0.15	-0.19*	-0.32*	-0.02	0.08	1.00	-0.52*	-0.87*
Protein content	-0.11	-0.05	0.13	-0.29*	-0.25*	-0.10	-0.04	-0.12	0.17	-0.52*	1.00	0.07
Moisture content	0.23*	-0.22*	-0.02	0.28*	0.32*	0.30*	0.38*	0.09	-0.16	-0.87*	0.07	1.00
pH_{24h}	0.01	0.07	-0.06	-0.20*	-0.27*	-0.23*	-0.15	-0.05	0.18	-0.08	0.30*	-0.05
T_{24h}[7]	-0.22*	0.08	0.11	-0.13	-0.19*	-0.22*	-0.11	0.00	0.02	0.23*	-0.10	-0.26*
pH_{7d}	0.05	-0.17	0.09	-0.10	-0.04	-0.21*	-0.04	-0.13	-0.02	0.08	-0.14	-0.06
Sarcomere length	-0.16	-0.21*	0.28*	0.07	0.02	0.02	0.15	0.15	-0.05	0.05	-0.14	0.04
Lightness	0.05	-0.09	0.03	-0.07	0.02	0.01	-0.20*	-0.14	0.17	0.10	0.09	-0.16
Chroma	0.31*	0.18	-0.38*	0.27*	0.31*	0.09	0.19*	0.02	-0.25*	0.11	-0.18	-0.06
Hue	0.03	-0.05	0.01	-0.12	-0.06	0.03	-0.25*	-0.17	0.27*	0.03	0.20*	-0.06
MMB[8]	0.33*	0.25*	-0.45*	0.52*	0.43*	0.22*	0.47*	0.21*	-0.43*	0.07	-0.29*	0.08
DMB[9]	-0.31*	-0.34*	0.50*	-0.37*	-0.33*	-0.18	-0.25*	-0.03	0.24*	-0.14	0.12	0.06
OMB[10]	0.19*	0.27*	-0.35*	0.16	0.17	0.10	0.05	-0.07	-0.06	0.13	0.00	-0.11

Purge loss	-0.19*	-0.14	0.26*	-0.01	0.00	0.08	-0.02	0.10	0.04	0.01	-0.08	0.07
Cooking loss	0.30*	-0.10	-0.16	0.24*	0.29*	0.26*	0.17	0.06	-0.20*	-0.17	0.03	0.18
Cooking time	0.16	-0.03	-0.10	-0.03	0.02	0.00	-0.24*	-0.09	0.00	0.13	0.06	-0.18
STD[11]	0.14	-0.18	0.03	0.18	0.17	0.18	0.22*	0.13	-0.21*	-0.05	-0.05	0.06

[1] Fibre distribution

[2] Slow oxidative muscle fibre

[3] Fast oxidative glycolytic muscle fibre

[4] Fast glycolytic muscle fibre

[5] Fibre cross-sectional areas

[6] Expressed as a percentage of total collagen content

[7] Temperature at 24 hours post mortem

[8] Metmyoglobin relative content

[9] Deoxymyoglobin relative content

[10] Oxymyoglobin relative content

[11] Standard deviation of shear force

*P < 0.05

Table 4-5b Pearson correlation coefficients between muscle and meat characteristics of the m. *gluteus medius*

Variable	pH_{24h}	T_{24h}[1]	pH_{7d}	Sarcomere length	Lightness	Chroma	Hue	MMB[2]	DMB[3]	OMB[4]	Purge loss	Cooking loss	Cooking time	STD[5]
SO%[6,7]	0.01	-0.22*	0.05	-0.16	0.05	0.31*	0.03	0.33*	-0.31*	0.19*	-0.19*	0.30*	0.16	0.14
FOG%[8]	0.07	0.08	-0.17	-0.21*	-0.09	0.18	-0.05	0.25*	-0.34*	0.27*	-0.14	-0.10	-0.03	-0.18
FG%[9]	-0.06	0.11	0.09	0.28*	0.03	-0.38*	0.01	-0.45*	0.50*	-0.35*	0.26*	-0.16	-0.10	0.03
SO area[10]	-0.20*	-0.13	-0.10	0.07	-0.07	0.27*	-0.12	0.52*	-0.37*	0.16	-0.01	0.24*	-0.03	0.18
FOG area	-0.27*	-0.19*	-0.04	0.02	0.02	0.31*	-0.06	0.43*	-0.33*	0.17	0.00	0.29*	0.02	0.17
FG area	-0.23*	-0.22*	-0.21	0.02	0.01	0.09	0.03	0.22*	-0.18	0.10	0.08	0.26*	0.00	0.18
Muscle weight	-0.15	-0.11	-0.04	0.15	-0.20*	0.19*	-0.25*	0.47*	-0.25*	0.05	-0.02	0.17	-0.24*	0.22*
Total collagen	-0.05	0.00	-0.13	0.15	-0.14	0.02	-0.17	0.21*	-0.03	-0.07	0.10	0.06	-0.09	0.13
Soluble collagen[11]	0.18	0.02	-0.02	-0.05	0.17	-0.25*	0.27*	-0.43*	0.24*	-0.06	0.04	-0.20*	0.00	-0.21*
Fat content	-0.08	0.23*	0.08	0.05	0.10	0.11	0.03	0.07	-0.14	0.13	0.01	-0.17	0.13	-0.05
Protein content	0.30*	-0.10	-0.14	-0.14	0.09	-0.18	0.20*	-0.29*	0.12	0.00	-0.08	0.03	0.06	-0.05
Moisture content	-0.05	-0.26*	-0.06	0.04	-0.16	-0.06	-0.06	0.08	0.06	-0.11	0.07	0.18	-0.18	0.06
pH_{24h}	1.00	0.14	-0.07	-0.18	0.03	-0.19*	0.12	-0.22*	0.13	-0.03	-0.20*	-0.10	-0.06	-0.28*
T_{24h}	0.14	1.00	0.02	-0.04	-0.06	-0.06	-0.13	-0.18	0.16	-0.09	0.14	-0.15	-0.18	-0.10
pH_{7d}	-0.07	0.02	1.00	0.09	0.12	0.04	-0.14	-0.05	0.27*	-0.29*	-0.13	-0.13	0.15	0.12
Sarcomere length	-0.18	-0.04	0.09	1.00	0.04	-0.03	-0.04	0.09	0.10	-0.16	0.19*	0.00	-0.10	-0.15
Lightness	0.03	-0.06	0.12	0.04	1.00	0.50*	0.73*	-0.44*	-0.11	0.36*	0.16	0.12	0.12	0.11
Chroma	-0.19	-0.06	0.04	-0.03	0.50*	1.00	0.10	0.25*	-0.58*	0.55*	0.14	0.29*	0.09	0.24*
Hue	0.12	-0.13	-0.14	-0.04	0.73*	0.10	1.00	-0.50*	-0.22*	0.52*	0.07	0.12	0.12	-0.08
MMB	-0.22*	-0.18	-0.05	0.09	-0.44*	0.25*	-0.50*	1.00	-0.53*	0.11	-0.17	0.19	0.11	0.18
DMB	0.13	0.16	0.27*	0.10	-0.11	-0.58*	-0.22*	-0.53*	1.00	-0.90*	0.05	-0.25*	-0.14	-0.09
OMB	-0.03	-0.09	-0.29*	-0.16	0.36*	0.55*	0.52*	0.11	-0.90*	1.00	0.02	0.20*	0.11	0.01

Purge loss	-0.20*	0.14	-0.13	0.19*	0.16	0.14	0.07	-0.17	0.05	0.02	1.00	-0.01	-0.12	0.03
Cooking loss	-0.10	-0.15	-0.13	0.00	0.12	0.29*	0.12*	0.19	-0.25*	0.20*	-0.01	1.00	0.58*	0.07
Cooking time	-0.06	-0.18	0.15	-0.10	0.12	0.09	0.12	0.11	-0.14	0.11	-0.12	0.58*	1.00	0.05
STD	-0.28*	-0.10	0.12	-0.15	0.11	0.24*	-0.08	0.18	-0.09	0.01	0.03	0.07	0.05	1.00

[1] Temperature at 24 hours post mortem

[2] Metmyoglobin relative content

[3] Deoxymyoglobin relative content

[4] Oxymyoglobin relative content

[5] Standard deviation of shear force

[6] Fibre distribution

[7] Slow oxidative muscle fibre

[8] Fast oxidative glycolytic muscle fibre

[9] Fast glycolytic muscle fibre

[10] Fibre cross-sectional area

[11] Expressed as a percentage of total collagen content

*$P < 0.05$

Table 4-6 Regression models for myofibrillar (MF-SF), connective tissue (CT-SF), and peak shear force (SF) as selected by the stepwise selection in the m. *semitendinosus*

Variable entered	Peak SF	MF-SF	CT-SF
1	Muscle weight[1] (0.3515)	Muscle weight (0.3034)	Muscle weight (0.3131)
2	FG%[2] (0.0472)	Cooking loss (0.1398)	FG% (0.0719)
3	Moisture content (0.0266)	pH_{24h} (0.0168)	Moisture content (0.0373)
4	OMB[3] (0.0026)	-	Total collagen (0.0188)
5	-	-	OMB (0.0122)
Model R-square	0.4366	0.4600	0.4533

[1] Variable (partial R-square)

[2] Fast glycolytic muscle fibre distribution

[3] Oxymyoglobin relative content

Table 4-7 Regression models for myofibrillar (MF-SF), connective tissue (CT-SF), and peak shear force (SF) as selected by the stepwise selection in the m. *gluteus medius*

Variable entered	Peak SF	MF-SF	CT-SF
1	Cooking loss (0.3073)	Cooking loss (0.4497)	Muscle weight (0.2880)
2	Muscle weight (0.1529)	Muscle weight (0.0669)	pH_{24h} (0.0772)
3	pH_{24h} (0.0358)	pH_{7d} (0.0157)	Cooking loss (0.0321)
4	pH_{7d} (0.0262)	pH_{24h} (0.0120)	FG%[2] (0.0195)
Model R-square	0.5222	0.5443	0.4168

[1] Variable (partial R-square)

[2] Fast glycolytic muscle fibre distribution

Figure 4-1 Example of a shear curve measured with a Texture Analyser

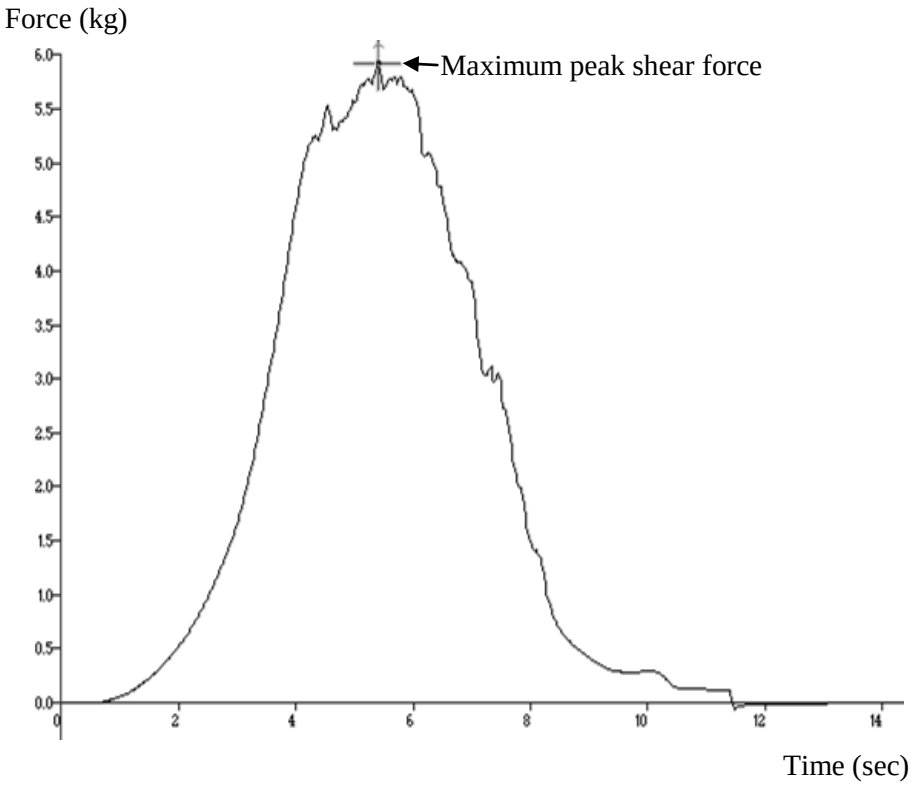
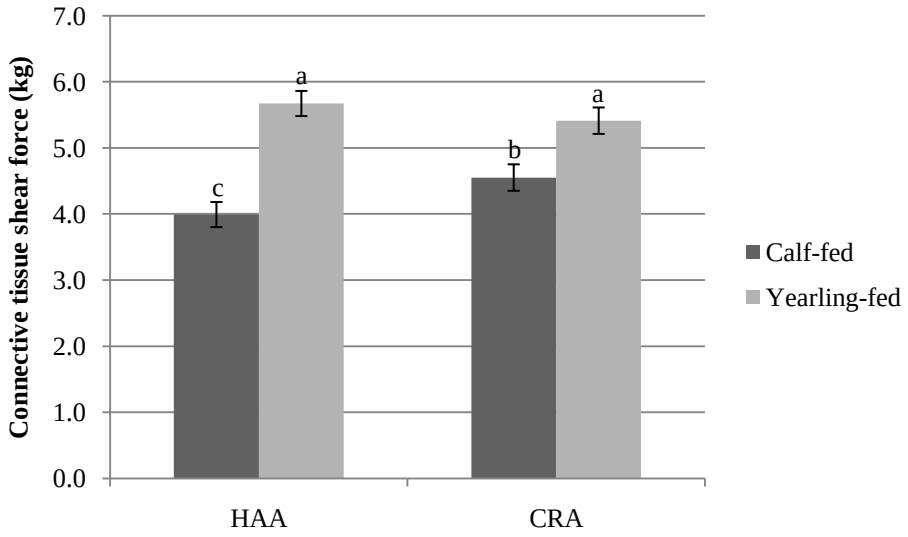


Figure 4-2 Connective tissue shear force as affected by age at slaughter and breed cross for the m. *gluteus medius*



[ab] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus

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Chapter 5

Summary and general conclusion

5.1 SUMMARY OF RESULTS

Hormonal growth implants and β-adrenergic agonist (β-AA) feed supplements have been used to increase growth rate and reduce time to market of beef cattle. Finishing steers at less than 18 months of age, with or without growth promotants, offers the advantages of reducing production costs, which increases profit per beef animal if meat yield and quality are satisfactory. Although beef producers gauge growth efficiency and profitability closely, they are usually not aware of consumer perceptions of the quality of the meat their cattle produce. Beef quality is regularly audited in Canada to estimate the retail quality of beef, with the regularity of the audits accounting for changes in animal genetics and production practices over time. Beef quality audits were held in Canada in 1995-1996 and in 1998-1999 (Van Donkersgoed et al., 2001; Van Donkersgoed et al., 1997) but these were plant audits only. The most recent Canadian beef quality audits (CCA, 2010) did include consumer satisfaction surveys because sustained meat industry profitability relies upon consumer eating satisfaction. Although results are not yet available, if consumers are concerned with the tenderness of the steaks they purchase at retail, this will indicate if beef quality requires improvement.

Recent research has indicated that growth promotants such as hormonal implants (Faucitano et al., 2008; Foutz et al., 1997) and β-AA (Avendaño-Reyes et al., 2006; Gruber et al., 2008; Strydom et al., 2009) can individually contribute to beef toughness, with the extent of toughening by these two classes of growth promotants potentially additive. This project intended to examine this possibility and describe the effect of different growth promotion systems on the meat quality of crossbred steers. The different treatments involved two ages at slaughter, 12 to 13 months and 18 to 20 months, the use of hormonal growth implants (IMP) or not (NOIMP), the supplementation of the finishing diet with the β-AA ractopamine hydrochloride (RAC) or not (NORAC), and two different crossbreds, Hereford-Aberdeen Angus (HAA) or Charolais-Red Angus

(CRA). Meat quality was assessed on the m. *semitendinosus* (ST; eye of round) and m. *gluteus medius* (GM; top sirloin), with the ST and the GM representing muscles that have high and moderate connective tissue contents (Ramsbottom and Strandine, 1948). These muscles were chosen as they were anticipated to toughen with animal age due to the prominent content of connective tissue and the formation of heat insoluble cross-links (Lepetit, 2008). To identify whether connective tissue or myofibrillar proteins were contributing to the toughness of cooked beef in this study, the shear force (SF) deformation curve was deconstructed into myofibrillar and connective tissue portions. Meat tenderness is of great importance to the meat industry and understanding how meat structure relates to it will assist with improving this product characteristic.

The results of the present study indicated that muscle yield was improved with IMP, CRA crossbred, and slaughtering steers at 18 to 20 months (Section 2.3.1). This increase in yield was generally accompanied by adverse effects on the quality of both muscles, specifically an increase in the cooked beef SF (Section 3.3.6). Most of the time, when an increase in SF was observed, mean muscle fibre cross-sectional areas increased and/or the soluble collagen content decreased (Section 3.4.1). The ST meat water-holding capacity, as evaluated by the purge and cooking losses, was more affected in yearling-fed, CRA crossbred, and implanted steers than the GM (Section 3.3.5). The amount of purge loss usually increased when mean muscle fibre cross-sectional areas or the frequency of the large FG fibres increased (Section 3.4.2). Indeed, the FG fibres may not be as effective at binding water molecules as the intermediate FOG and small SO fibres because they have a reduced water:protein ratio (Geesink et al., 1993; Ryu and Kim, 2006).

Hormonal growth implants are known to increase the leanness of meat (Singh et al., 2006), which corresponded to the decreased fat and increased moisture contents observed in the GM, and the trend towards reduced fat content in the ST with growth implant treatment (Section 2.3.2). Charolais cattle are also known for their high lean:fat ratio and the Angus and Hereford cattle for their low to moderate lean:fat ratio (Buchanan and Dolezal, 1999). The variations observed in this study were in accordance with that expectation because the fat content was lower and both the water and protein contents were greater in the muscles from the carcasses of the CRA steers than in those from the carcasses of the HAA steers (Section 2.3.2). Changes occurring to the meat mean muscle

pH and temperature values at 24 hours and pH at seven days post mortem (Section 3.3.1), objective colour measurements (Section 3.3.2), myoglobin relative contents (Section 3.3.3), and sarcomere lengths (Section 3.3.4) were limited, suggesting that production management practices had little if any effect on these muscle characteristics.

The growth promotant RAC did not increase muscle weight or change the muscle composition but it was related to increased meat purge loss (Section 3.3.5), which is not desirable because it reduces saleable weight (Offer and Cousins, 1992) and diminishes consumer acceptance (Cheng and Sun, 2008). Even though this growth promotant had little effect on muscle weights in this study, histological changes occurred (Section 2.3.3) that may have been responsible for the great decrease in the meat water-holding capacity. As explained with IMP, the muscle fibre shift from the small SO to the large FG fibres observed in the GM with RAC supplementation may have caused the water:protein ratio to be higher than usual. The water molecules were then probably not effectively retained by the protein, causing drip loss (Geesink et al., 1993; Ryu and Kim, 2006). In contrast to the results of Bass et al. (2009) and Winterholler et al. (2008), IMP and RAC did not have a synergistic effect on muscle growth but affected the FG muscle fibre mean cross-sectional areas synergistically as observed in a three-way interaction for the ST. With IMP and RAC increasing FG fibre size, which are the largest muscle fibres, muscle weight may be improved with additional time on RAC and beef producers could benefit from the enhanced muscle yield. The interaction between RAC, age at slaughter, and breed cross on the FG and FOG fibre mean cross-sectional areas of the ST suggested that feeding RAC in accordance with the growth patterns of the steers and their individual muscles could potentially lead to an overall increase of muscle weight, although that was not observed.

Changes in SF occurring in the ST were more related to the connective tissue component than they were in the GM, whereas in the GM, variations in SF were associated with the myofibrillar component more than in the ST. Meat characteristics representing the state of the myofibres, such as cooking loss and pH, were good predictors of SF in the GM muscle. Muscle weight also explained a portion of the variation in SF in the GM and it was this characteristic that best predicted SF in the ST. Therefore, as muscle weight increased, SF increased as well. These observations matched with concomitant increase in muscle fibre cross-sectional areas and/or decrease in

collagen solubility. These changes seemed age related, but whether muscle weight is related to SF in steers older than 18 to 20 months remains to be confirmed.

5.2 IMPLICATIONS FOR MEAT SCIENCE AND BEEF INDUSTRY

Using management systems that offer the possibility to reduce the time required to finish a steer for market can be beneficial to the beef producers. Indeed, reducing variable costs related to the feed, yardage, bedding, manure hauling, and labour time will increase the benefit per beef animal and provide better financial return to the beef producers. For instance, costs associated with yardage, including buildings, machinery, and labour, were estimated at $0.67/head/day (Kaliel, 2004). Also, costs associated with feeding barley, barley silage, and supplements were estimated at $1.02/day (AAMS, 2000). Reducing these costs will allow beef cattle producers to remain competitive in the global beef commodity market. Also, enhancing the growth rate of steers without affecting cooked meat tenderness, as seen with IMP in the GM, meets both the beef producer and consumer expectations.

This project provided more information on the use of both hormonal implants and RAC on meat characteristics and quality from crossbred steers. Few studies have given attention to the effects of different RAC feeding and implantation strategies and none addressed subsequent meat quality (Bass et al., 2009; Sissom et al., 2007; Winterholler et al., 2008). Furthermore, this study contributed to understanding the biological differences between muscles within an animal because the ST and the GM have different fibre patterns (Kirchofer et al., 2002) and connective tissue contents (Ramsbottom and Strandine, 1948). Therefore; this study offered the possibility of measuring the impact of RAC and IMP on these muscles by quantifying the action of the different treatments and clarifying the effects of the muscle fibres and the collagen contents on meat tenderness.

Slaughtering steers at 12 to 13 months of age increased and tended to increase the soluble collagen content in the GM and the ST respectively (Section 2.3.4), thereby reducing the contribution of the heat insoluble fraction to the cooked meat SF by decreasing the time that heat-stable collagen cross-links have to form in the live animal (Purslow, 2005). Reducing the number of heat-stable cross-links in beef muscle may have the greatest impact on muscles that have moderate connective tissue content, meaning

that these muscles may be too tough to pan-fry when harvested from a steer that is 18 to 20 months old, but may be of acceptable quality when from a steer of 12 to 13 months old. The results of this study supported this conclusion and indicated that different meat cuts could have increased value potential when harvested from young steers, which could increase the economic return of a carcass.

5.3 SUGGESTIONS FOR FURTHER RESEARCH

RAC was shown to improve the final body weight of steers or heifers in other studies (Avendaño-Reyes et al., 2006; Gruber et al., 2007; Walker et al., 2007; Winterholler et al., 2007) but did not affect muscle weight in the present study. Understanding the effect of RAC on muscle growth could be further investigated by an examination of the binding of ractopamine to β-adrenergic receptors (AR). Indeed, as to whether ractopamine acts through the β1- and/or the β2-AR has yet to be determined as studies are contradictory (Winterholler et al., 2008; Winterholler et al., 2007) and this may be key as to the extent to which RAC improves muscle growth and final weight. The administration of RAC was also associated with an increase in raw meat purge loss, the occurrence of which was not easily explained. The water-binding capacity of different muscle fibre types by adjusting for their mean cross-sectional areas and their mean fibre distribution could be further investigated.

Because RAC did not increase muscle weights in this study, IMP and RAC did not seem to have had synergistic effects on the growth of the muscles studied. IMP and RAC may have had a synergistic effect in muscles that were not analyzed in this study, as observed in other studies on the m. *longissimus* (Bass et al., 2009; Gonzalez et al., 2007). Characterizing which muscles are most affected in the carcass by IMP and RAC should be a priority for the beef industry so that the advantages of this management strategy are fully understood. The possibility of antagonist effects between these two growth promotants is not excluded, but the study performed by Bass et al. (2009) did not identify this relationship. Indeed, different muscles have different growth pattern (Berg and Butterfield, 1976) and can be affected to different extents by growth promotants. Moreover, the changes in fibre pattern due to growth promotants should be different because RAC and hormonal implants use different metabolic pathways of action (Aberle et al., 2001; Hossner, 2005; Rehfeldt et al., 2004).

Another area that could be further explored is the relationship between muscle weight and the cooked meat SF in steers older than 20 months. The size of muscle fibres (Crouse et al., 1991) and the amounts of total and soluble collagen (Aberle et al., 2001) are known to affect meat tenderness. Also, the toughness of beef is known to increase with age (Shorthose and Harris, 1990). Nevertheless, we do not know if muscle weight could be representative of the beef meat tenderness after 20 months of age knowing that growth stops at some point during the steer life time and protease activities vary between growing and mature steers. So, it would be interesting to know if peak SF, MF-SF, and CT-SF continue to be related to the same extent to muscle weight in animals older than 20 months.

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APPENDIX

A-1 Effect of age at slaughter, implantation strategy, ractopamine hydrochloride feed supplementation, and breed cross on temperature, objective coluor measurements, and myoglobin relative contents of the m. *gluteus medius* (Table A-1)

A-2 Effect of age at slaughter (A), implantation strategy (I), ractopamine hydrochloride feed supplementation (R), and breed cross (B) on temperature, objective colour measurements, and myoglobin relative contents of the m. *semitendinosus* (Table A-2)

A-3 Mean chroma at 24 hours post mortem of the m. *semitendinosus* as affected by an interaction between implantation strategy and age at slaughter (Figure A-1)

A-4 Mean metmyoglobin relative content at 24 hours post mortem of the m. *semitendinosus* as affected by an interaction between implantation strategy and age at slaughter (Figure A-2)

A-5 Mean chroma at 24 hours post mortem of the m. *semitendinosus* as affected by an interaction between ractopamine hydrochloride feed supplementation and breed cross (Figure A-3)

A-6 Mean metmyoglobin relative content at 24 hours post mortem of the m. *semitendinosus* as affected by an interaction between ractopamine hydrochloride feed supplementation and breed cross (Figure A-4)

Table A-1 Effect of age at slaughter, implantation strategy, ractopamine hydrochloride feed supplementation, and breed cross on temperature, objective colour measurements, and myoglobin relative contents of the m. *gluteus medius*

Variables	Age at slaughter		Implantation strategy		Ractopamine feeding		SEM[5]	Breed cross		SEM
	Calf-fed	Yearling-fed	NOIMP[1]	IMP[2]	NORAC[3]	RAC[4]		HAA[6]	CRA[7]	
n=	56	56	56	56	56	56	-	64	48	-
24 hours post mortem										
Lightness (L*)	39.33[a]	37.19[b]	38.19	38.33	37.91	38.61	0.35	38.49	38.03	0.33
Chroma (%)	25.31[b]	27.46[a]	26.35	26.42	26.29	26.48	0.28	26.77[a]	26.00[b]	0.27
Hue angle (°)	35.45[a]	33.52[b]	34.33	34.64	34.33	34.64	0.26	34.44	34.53	0.24
MMB[8,9]	0.17[b]	0.20[a]	0.19	0.18	0.19	0.18	0.00	0.18	0.18	0.00
DMB[10]	0.15[a]	0.12[b]	0.13	0.13	0.13	0.13	0.01	0.13	0.13	0.01
OMB[11]	0.68	0.69	0.68	0.68	0.68	0.69	0.01	0.68	0.68	0.01
7 days post mortem										
Temperature	2.82	2.63	2.76	2.69	2.89	2.56	0.20	2.73	2.72	0.14

[1] No hormonal implant

[2] Hormonal implants

[3] No ractopamine hydrochloride feed supplementation

[4] Ractopamine hydrochloride feed supplementation

[5] Pooled standard error of the mean (SEM) for age at slaughter, implantation strategy, and ractopamine feed supplementation

[6] Crossbred Hereford-Aberdeen Angus

[7] Crossbred Charolais-Red Angus

[8] Summation of MMB, DMB, and OMB relative contents within a treatment effect may not equal 1.00 due to number rounding [9] Metmyoglobin relative content

[10] Deoxymyoglobin relative content

[11] Oxymyoglobin relative content

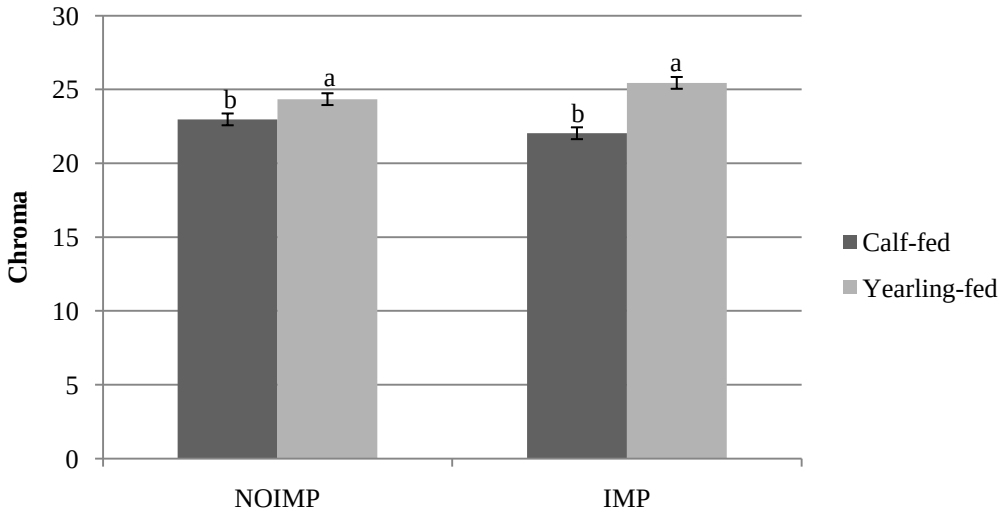
[ab] Means within the same row within a main effect with different superscript letters are significantly different ($P < 0.05$)

Table A-2 Effect of age at slaughter (A), implantation strategy (I), ractopamine hydrochloride feed supplementation (R), and breed cross (B) on temperature, objective colour measurements, and myoglobin relative contents of the m. *semitendinosus*, including significant interactions

Variables	Age at slaughter		Implantation		Ractopamine feeding			Breed cross			Interaction	
	Calf-fed	Yearling-fed	NOIMP[1]	IMP[2]	NORAC[3]	RAC[4]	SEM[5]	HAA[6]	CRA[7]	SEM	AxI	RxB
n=	56	56	56	56	56	56	-	64	48	-	-	-
24 hours post mortem												
Lightness (L*)	43.56[a]	41.01[b]	42.39	42.19	42.39	42.19	0.35	42.18	42.39	0.32	NS[8]	NS
Chroma (%)	22.50[b]	24.89[a]	23.66	23.73	24.01	23.38	0.28	23.75	23.64	0.28	*[9]	*
Hue angle (°)	40.97[a]	37.93[b]	39.25	39.65	39.33	39.57	0.26	39.28	39.63	0.27	NS	NS
MMB[10,11]	0.11[b]	0.15[a]	0.13	0.14	0.14[a]	0.13[b]	0.00	0.13	0.13	0.00	*	*
DMB[12]	0.23[a]	0.19[b]	0.21	0.21	0.2	0.22	0.01	0.21	0.21	0.01	NS	NS
OMB[13]	0.66	0.66	0.66	0.66	0.66	0.66	0.00	0.65	0.66	0.01	NS	NS
7 days post mortem												
Temperature	2.74	2.59	2.7	2.64	3.01[a]	2.32[b]	0.19	2.7	2.64	0.13	NS	NS

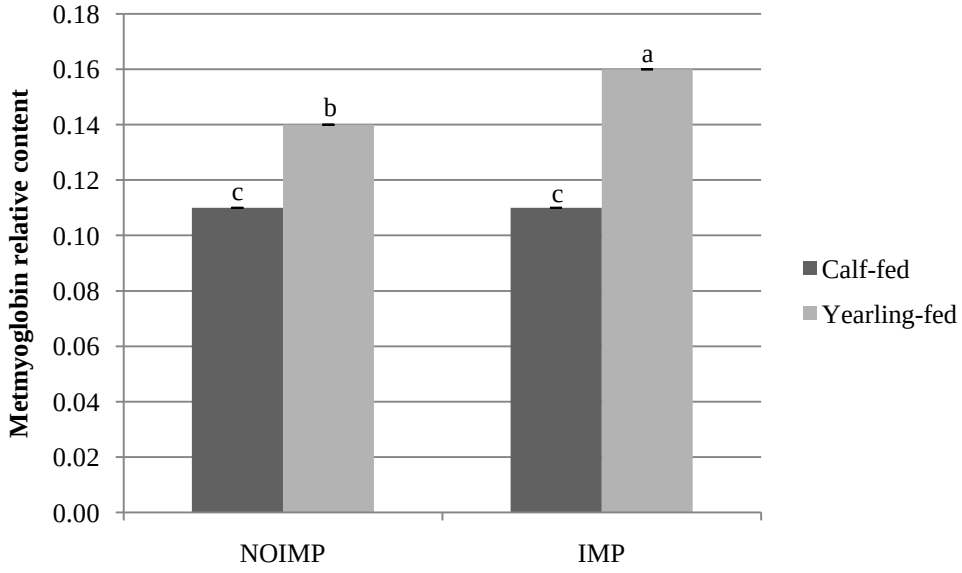
[1] No hormonal implant
[2] Hormonal implants
[3] No ractopamine hydrochloride feed supplementation
[4] Ractopamine hydrochloride feed supplementation
[5] Pooled standard error of the mean (SEM) for age at slaughter, implantation strategy, and ractopamine feed supplementation
[6] Crossbred Hereford-Aberdeen Angus
[7] Crossbred Charolais-Red Angus
[8] Non significant
[9] Significant at ($P < 0.05$)
[10] Summation of MMB, DMB, and OMB relative contents within a treatment effect may not equal 1.00 due to number rounding
[11] Metmyoglobin relative content
[12] Deoxymyoglobin relative content
[13] Oxymyoglobin relative content
[ab] Means within the same row within a main effect with different superscript letters are significantly different ($P < 0.05$)

Figure A-1 Mean chroma value at 24°hours post mortem of the m. *semitendinosus* as affected by an interaction between implantation strategy and age at slaughter



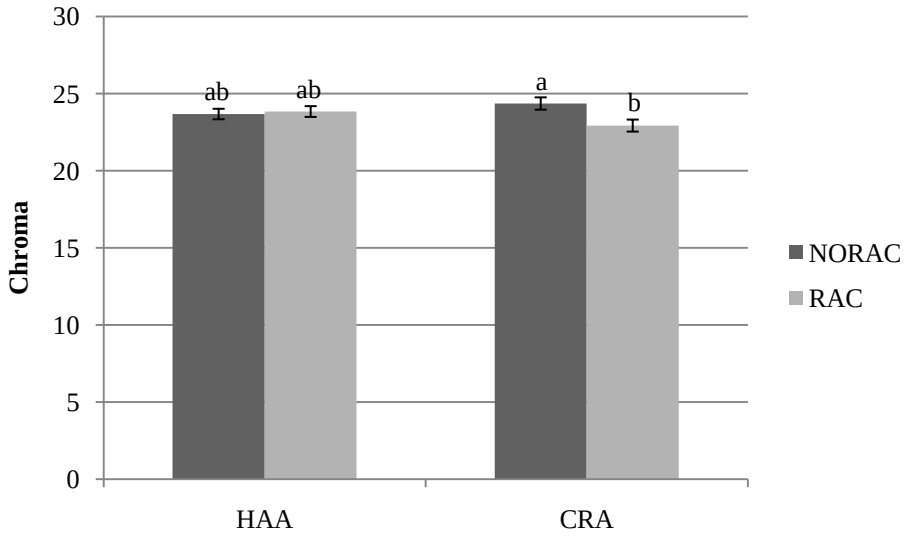
[ab] Columns with different letters are significantly different (P < 0.05)
Error bars are standard error of the mean (SEM)
NOIMP = No hormonal implant; IMP = Hormonal implants

Figure A-2 Mean metmyoglobin relative content at 24°hours post mortem of the m. *semitendinosus* as affected by an interaction between implantation strategy and age at slaughter



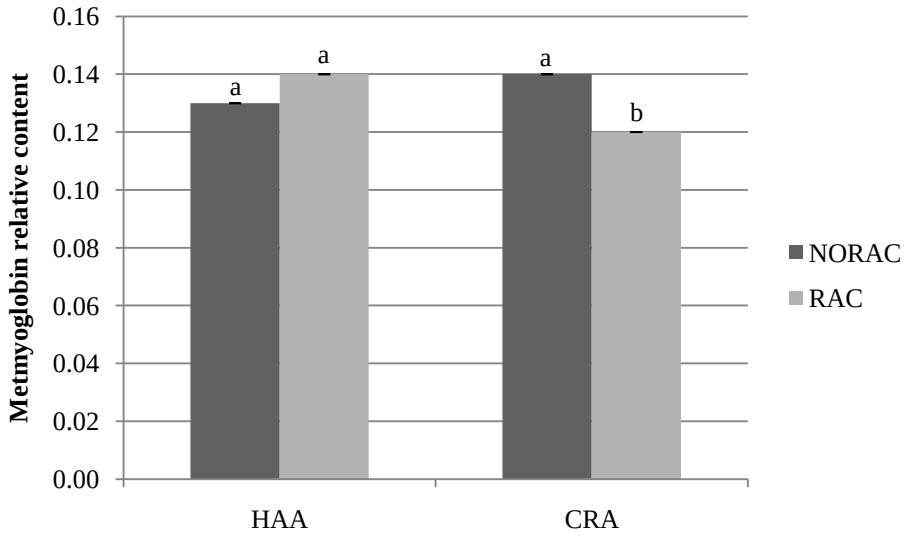
[abc] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
NOIMP = No hormonal implant; IMP = Hormonal implants

Figure A-3 Mean chroma value at 24°hours post mortem of the m. *semitendinosus* as affected by an interaction between ractopamine hydrochloride feed supplementation and breed cross



[ab] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
NORAC = No ractopamine hydrochloride feed supplementation; RAC = Ractopamine hydrochloride feed supplementation; HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus

Figure A-4 Mean metmyoglobin relative content at 24°hours post mortem of the m. *semitendinosus* as affected by an interaction between ractopamine hydrochloride feed supplementation and breed cross



[ab] Columns with different letters are significantly different ($P < 0.05$)
Error bars are standard error of the mean (SEM)
NORAC = No ractopamine hydrochloride feed supplementation; RAC = Ractopamine hydrochloride feed supplementation; HAA = Crossbred Hereford-Aberdeen Angus; CRA = Crossbred Charolais-Red Angus