MUSCLE PROFILING: INVESTIGATION OF THE *M. Rhomboideus* DERIVED FROM *Bos indicus* INFLUENCED CATTLE

A Thesis

by

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ABSTRACT

Research has shown the *M. Rhomboideus* (Rho) from purebred Nellore (*Bos indicus*, BI) carcasses to be unique in its proximate chemical composition, indicated by increased lipid deposition. Thus, potential for the deposition of mono- (MUFA) and poly-unsaturated (PUFA) fatty acids exists. The objective of this study was to assess the fatty acid composition of the lean portion and subcutaneous fat (SQF) of the Rho from BI-influenced cattle. Three replications of 4 USDA Choice (Ch) and 4 USDA Select (Se) Rho muscles were selected (n = 24). Total MUFA, PUFA, saturated fatty acid (SFA) percentages, and MUFA:SFA ratio of lean portion of the Rho muscle were not different ($P > 0.05$) between Ch Rho muscles (41.47, 7.17, 47.12%, 0.89, respectively) and Se Rho muscles (42.97, 6.34, 50.69%, 0.86, respectively). However, mean averages across both quality grades (QG) for total MUFA, PUFA, SFA, and MUFA:SFA (42.19, 6.76, 48.91%, 0.88, respectively) in the lean portion of the Rho were similar to reported fatty acid averages of *Bos taurus* (BT) *longissimus dorsi* lean portion (47.81, 4.35, 47.83%, 1.0, respectively). The total MUFA percentage (49.35%) and MUFA:SFA ratio (1.07) in Ch SQF was significantly higher ($P < 0.001$) compared to Se SQF (45.40%, 0.92, respectively). Total PUFA (4.33 vs 3.69%) and SFA percentages (50.27 vs 46.95%) in Se SQF were higher ($P < 0.02$) compared to Ch SQF. Mean averages across both QG for total MUFA, PUFA, SFA, and MUFA:SFA (47.38, 4.01, 48.61%, 1.00, respectively) in Rho muscle SQF were similar to reported FAC averages of BT brisket SQF (56.58, 2.53, 35.87% 1.47, respectively). The Rho muscle poses potential, due to availability and fatty acid composition to be marketed as product that is beneficial to human health.

DEDICATION

To my niece and goddaughter, Emmet Louise Valenta, whose life lies ahead of her.

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CHAPTER I

INTRODUCTION AND

LITERATURE REVIEW

1.1 Introduction

Extensive work has been done to assess the myology, composition, and quality in *Bos taurus* (BT) carcasses. However, assessment of individual muscles derived from *Bos indicus* (BI) influenced cattle is limited. Due the number of crossbred beef operations in the U.S., one can reasonably conclude that there is a large number of pounds of BI x BT meat in the marketplace. The *M. Rhomboideus* (Rho) is the most identifiable muscle of Brahman (BI) cattle, and thus a major muscle of BI influenced cattle. The Rho muscle could possibly be a sustainable source of unrealized BI x BT products. The Rho muscle accounts for approximately 1.0% of chilled carcass weight in cattle that are purebred Nellore (BI) (Pedrão et al., 2011). In the U. S. market, the dorsal portion of the Rho muscle from heavily influenced or purebred BI carcasses is removed (IMPS 116A, USDA, 2014). This meat is used in ground beef production. The Rho muscle is not commonly used as a wholesale cut in the U.S. because consumers are not accustomed to the purchase of this cut in a retail setting. However, as indicated by Pedrão et al. (2011), in Brazilian markets, the Rho muscle is often utilized as a grilled or barbecued item ("cupim") and is considered a delicacy. If the Rho muscle's attributes were better understood, there is potential for the Rho to be marketed as a product that offers benefits to human health or an alternative to brisket.

1.2 Beef Muscle Composition

Beef muscle is composed primarily of lean, fat, water, and connective tissue. The biochemical properties of each component have been shown to affect the ability of a muscle to be utilized as steaks, roasts, or in processed products (Kerry et al., 2002). The interrelation of each component impacts overall meat quality and consumer desirability.

Ang et al. (1984) reported that protein, fat, and moisture account for approximately 99% of the weight of animal tissues. Carbohydrates and trace amounts of inorganic constituents form the remaining one percent (ash), which is relatively consistent across species and sub species (Ang et al., 1984; Aberle et al., 2012).

1.1.1 Protein

Proteins form the structures of muscles and are the functional mechanisms that create movement. The most abundant protein found in beef skeletal muscle is myosin. Myosin and actin are arranged into a myofilament. The myofilament and structural proteins are organized into a lattice structure to form the sarcomere, the smallest contractile unit of a muscle fiber (Kerry et al., 2002; Listrat et al., 2016).

A study of proximate composition reported that the percent protein in the brisket, flat half, from BT was 21.7 and 21.5% for USDA Choice (Ch) and USDA Select (Se), respectively (Wahrmund-Wyle et al., 2000). Protein percentage of beef muscle varies due to many external factors, however it is thought that myosin heavy chain (MHC) isoforms are the major influencers of protein content (Hunt and Hedrick, 1977). Due to

the differences in the MHC isoforms, there are differences in the intracellular (non-collagenous proteins) and extracellular fibers (collagen) (Woessner, 1962).

1.1.2 Moisture

Water (moisture) exists in the muscle fibers as a means to transport nutrients to and throughout cells. Protein content and pH are related to water-holding capacity (WHC, moisture) in postmortem meat. Postmortem, muscle fibers shrink expelling moisture into intracellular spaces. This water is expelled at the cut ends of muscles and lost through evaporation (Guignot et al., 1993). The extent of muscle fiber shrinkage is impacted by muscle pH (Kerry et al., 2002). As the pH of a carcass declines towards the isoelectric point of muscle proteins (pH = 5.2), the negative electrostatic repulsion between myofilaments is reduced (Kerry et al., 2002). Thus, water is driven from the muscle interior. According to Ryu and Kim (2005), a greater overall decline in pH was found in muscles that depended heavily on glycolysis for energy metabolism (α-white fibers). Muscles with a greater proportion of α-white fibers result in a greater loss of moisture due to increased lactate production postmortem, which results in a lower ultimate pH. Wahrmund-Wyle et al. (2000) reported that the brisket, flat half, from BT cattle contained 74.4 and 74.3% moisture in Ch and Se, respectively.

1.1.3 Fat

Fat or adipose tissue can be deposited at four locations. Fat locations and chronological order of deposition are: viscerally (kidney, pelvic, and heart; KPH), subcutaneously (external), intermuscularly (seam), and finally intramuscularly (IMF;

marbling). Marbling is of particular importance as it is the basis for which producers receive premiums. Marbling is deposited mainly in the perimysium between muscle fiber bundles (Nishimura, 2010). Additional sensory attributes that marbling impacts are lower resistance when chewing, lubrication of myofilaments, disruption in connective tissue and myofibrils, and protection of excessive protein denaturation of meat that is cooked to higher degrees of doneness (Aberle et al., 2012).

Percentage of fat from BT brisket, flat half, reported by Wahrmund-Wyle et al. (2000) was 5.5 and 3.9% for Ch and Se, respectively. Studies have compared the relationship between beef muscle fiber types and indicate that marbling is typically positively correlated with the percentage of oxidative fibers (β-red fibers) and negatively with glycolytic fibers (α-white fibers) (Cassens and Cooper, 1971; Hwang et al., 2010). Additionally, IMF fat deposition has been shown to increase when cattle are finished on grain-fed diets (Suzuki et al., 1976; Rule et al., 1987; Mandell et al., 1998). Grain-finishing also changes the chemical composition of fat by increasing the amount of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) (Mandell et al., 1998; Poulson et al., 2004; Gama et al., 2013). This is important when considering human health and the consumption of red meat(s).

1.1.4 Fatty Acid Composition

Triglycerides, the basis of lipids, are composed of a glycerol backbone with three attached fatty acids. These fatty acids are present in two forms: saturated (no double bonds) or unsaturated (one or more double bond(s)). In beef, the most prevalent fatty

acid is MUFA oleic acid (18:1). However, high proportions of saturated fatty acids (SFA), such as palmitic (16:0) and stearic acid (18:0) are also present (Aberle et al. 1975).

The bulk of fatty acids are located in adipose tissue, with oleic acid being the most abundant MUFA (Wood et al., 2007; Turk and Smith, 2009). Historically, research efforts have been spent assessing the fatty acid composition of each fat depot, because each has a unique fatty acid profile. However, emphasis is placed more on muscle fatty acids, where fatty acids are present in the phospholipids of cells (Listrat et al., 2016). Fatty acids present in muscle, especially n-3 fatty acids with more than 20 carbons, are incorporated into phospholipids in ruminants (Listrat et al., 2016). Nevertheless, MUFA and PUFA fatty acids that come from triglycerides in adipose tissue or phospholipids in muscle are thought to be a benefit human nutrition. Besides human nutrition, fatty acids are essential to the development of sensory attributes such as flavor (Poulson et al., 2004).

Extensive research has been conducted to optimize feeding strategies that decrease the amount of SFA and increase MUFAs and PUFAs (Rule et al., 1987; Zembayashi and Nishimura, 1996; Mandell et al., 1998; Poulson et al., 2004; Wood et al., 2007; Gama et al., 2013; Shingfield et al., 2013). However, fatty acid profiles are also dependent on breed, anatomical location of muscle, and animal diet (Mandell et al., 1998; Turk and Smith, 2009; Shingfield et al., 2013; Listrat et al., 2016).

A human diet low in total fat and SFA cholesterol is recommended for lowering cholesterol (Grundy, 1989). Understanding fatty acid composition research stems from a trend of consumers becoming more health conscious and moving away from cuts with higher visible fat, while demanding products with higher nutritional value (Turk and Smith, 2009). It is generally accepted that MUFA and PUFA are a benefit to human health (De Smet et al., 2004; Listrat et al., 2016). It has been reported that MUFAs and PUFAs have the ability to increase high-density lipoprotein (good) cholesterol (HDL) (Mensink et al., 2003) and decrease low-density lipoprotein (bad) cholesterol (LDL) (Mensink and Katan, 1989).

1.1.5 Connective Tissue and Collagen

The purpose of connective tissue is to hold various parts of the body together. Connective tissue proteins are found throughout a carcass and are components of tendons, ligaments, fat, blood vessels, and muscles as epimysium, perimysium, and endomysium. Connective tissue structures are composed of three fiber types: collagen, elastin, reticulin; with collagen being the most abundant (Purslow, 2005). A general overview of connective tissue structures is: (1) endomysium is the thin connective tissue layer separating individual muscle fibrils, made of a random network of collagen fibers; (2) Perimysium is the connective tissue layer that surrounds and separates muscle bundles, as two parts: large (primary) fascicles and smaller (secondary) fascicles. Collagen fibers in the perimysium are organized in a two-set crossed-ply arrangement (Rowe, 1981). Perimysium accounts for 90% of total connective tissue in muscles

(Mccormick, 1999); and finally, (3) Epimysium is the connective tissue layer that delineates and separates individual muscles.

Collagen is the most important connective tissue fiber type in relation to tenderness and is comprised of tropocollagen molecules that overlap each other. At the base of these tropocollagen molecules is a sequence of amino acids, with about one third of the sequence being glycine (Aberle et al., 2012). Collagen molecules also possess a unique amino acid, hydroxyproline (HPP). Hydroxyproline may be used to measure total collagen content and solubility in muscle (Woessner, 1962). Total collagen content of beef can vary from 1% to 15% dry weight (Purslow, 2005). Collagen types vary in abundance and importance to muscle structure, however, types I, III, IV, V, VI, XII, and XIV have been identified in connective tissue (Listrat et al., 1999; Listrat et al., 2000). Types I and III are the major fiber-forming types in endomysium, perimysium, and epimysium (Purslow, 2005).

It has been demonstrated that collagen concentrations and solubility vary between muscles (Herring et al., 1967). Collagen variability and solubility leads to variation in tenderness of beef muscles (Mitchell et al., 1928; Ramsbottom et al., 1945). Muscles that are generally used for locomotion usually have greater amounts of connective tissue. These locomotive muscles are tougher compared to support muscles, which have less amounts of connective tissue (Ramsbottom et al., 1945; Melton et al., 1974a; Melton et al., 1975; Calkins et al., 1981).

1.2 Impacts on Sensory Attributes

1.2.1 Muscle Fiber Type

In beef cattle, it is known that lean, fat, and connective tissue percentage differences exist between carcasses. These differences are in part explained by muscle fiber types. Muscle fibers are characterized by their contractile and metabolic properties (Listrat et al., 2016). The contractile properties of muscle fibers are dependent on myosin heavy-chain isoforms (MHC) of the thick filaments (Listrat et al., 2016). Four types of MHC exist in mature beef cattle: I, IIa, IIx, IIb. The difference between these MHC isoforms is related to the activity of ATPase, which influences the speed of contraction. Type I fibers express low-intensity contractions and are resistant to fatigue. While, types IIa, IIx, and IIb exhibit vigorous contractions but fatigue easily. Fiber types develop in cattle based on physical and biochemical demands that are the result of energy metabolism in live muscle (Hocquette et al., 1998). Two pathways are used by muscles to metabolize energy (ATP): oxidative or glycolytic. Dependence on one of the two pathways determines the MHC isoforms. In the oxidative pathway, pyruvate is oxidized in the mitochondria through the citric acid cycle. Oxidative fibers, also known as β-red fibers, rely on myoglobin to carry oxygen to the cell to be used in tricarboxylic acid cycle. Myoglobin contributes to the red pigmentation observed in oxidative muscle fiber types. In the glycolytic pathway in living muscle, pyruvate is converted to lactic acid in the sarcoplasm. Glycolytic fibers, also known as α-white fibers, contain much less myoglobin due to limited need of oxygen and to metabolize energy. This reduction in

myoglobin content leads to less pigmentation. Based on the physiological and energy demand of a muscle (via external stimulus), fibers can be recruited to become more dependent on one pathway: I↔IIa↔IIx↔IIb (Listrat et al., 2016).While muscle fibers may be recruited, fibers are never fully converted to a different MHC isoform and will revert to the original isoform if the stimulus ceases.

Genetics, or breed, is a major determining factor of fiber types present in muscles (Johnston et al., 1981; Marshall, 1994). Melton et al. (1974, 1975) and Calkins et al. (1981) stated that muscles with increased α-white fibers have more connective tissue, less IMF fat, and are less tender than muscles with more β-red fibers. Whipple et al. (1990) concluded that animals that were 3/8 BI-influence had significantly more α-white fibers in the *longissimus dorsi* than BT carcasses. However, it has been reported that within a specific muscle, MHC isoform expression is influenced by animal sex (Johnston et al., 1975), nutrition (Suzuki et al., 1976; Mandell et al., 1998; De Smet et al., 2004) and maturity (Hiner and Hankins, 1950; Goll et al., 1963; Hunsley et al., 1971; Cornforth et al., 1980).

1.2.2 Color and Appearance

Meat color affects initial consumer decision to purchase a cut, and is influenced by the amount and chemical state of myoglobin. Muscles with high myoglobin containing fibers (type I and IIa) result in a positive relationship meat color, resulting in a more intense red color. Under vacuum conditions, myoglobin is in a reduced state and exhibits a purplish-color. When exposed to oxygen, a bright-cherry red color forms because of

myoglobin being oxygenated. Prolonged exposure to oxygen or denaturation of myoglobin with age results in an unattractive brown color (metmyoglobin). Many factors affect ante- and postmortem meat color: such as breed; sex; age; anatomical location; and physiological function of muscles; physical activity; pH and rate of pH decline; carcass chilling rate; and packaging conditions (Lebret et al., 2015). Moisture content of a cut may also influence color. Refraction and reflection of light from moisture within and on the surface of a cut results in differences in hue, chroma, and value (Swatland, 2012)

1.2.3 Tenderness

Tenderness and its variability is the most important sensory attribute that affects consumer acceptability (Whipple et al., 1990b; Crouse et al., 1993; Riley et al., 2005). In cattle, the relationship between muscle fiber characteristics and tenderness is complex and varies according to muscle, sex, age, and breed (Ellies-Oury et al., 2012). Additionally, collagen content and solubility is a strong influencer of tenderness (Eilert and Mandigo, 1993; Mccormick, 1999; Purslow, 2005).

1.2.4 Juiciness and Flavor

Flavor and juiciness are often highly correlated with tenderness by consumers (Huff-Lonergan et al., 2016). However, juiciness and flavor are impacted by proximate composition rather than muscle fiber type. Juiciness values result from the amount of moisture retained in meat post-cooking (Huff-Lonergan et al., 2016). This is because the majority of moisture and weight is lost through evaporation (Huff-Lonergan et al.,

2016). Beef flavor is strongly influenced by presence and composition of fatty acids in a meat (Wood et al., 2007), especially conjugated linoleic and oleic acid (Poulson et al., 2004). It is generally accepted that lower levels of marbling in meat results in a dry product with little taste (Listrat et al., 2016). Beef flavor is correlated to protein content in terms of physical muscle fiber types. Red muscle fiber types often have higher concentrations of myoglobin which result in more serum-like bloody aromatics and metallic flavors (Joo et al., 2013).

1.3 Comparison between Bos indicus and Bos taurus muscle

Breed type is a major factor affecting beef muscle composition. *Bos indicus* cattle have been reported to have: (1) reduced protein degradation and myofibrillar fragmentation (MF) (Goll et al., 1983; Koohmaraie et al., 1990), (2) differences in distribution of fat depots (Charles and Johnson, 1976), (3) fatty acid composition within depots (Huerta-Leidenz et al., 1993; Turk and Smith, 2009; Dinh et al., 2010), (4) and differences in amount of collagen/connective tissue compared to BT cattle. The combination of these attributes results in BI carcasses having increased Warner-Bratzler shear (WBS) force values and decreased tenderness (Wheeler et al., 1994). However, Wheeler et al. (1994) reported that variability of juiciness and flavor were not affected by breed differences.

1.3.1 Protein Degradation

Tenderness is the predominant quality determinant and sensory attribute related to consumer acceptability (Weir, 1960; Lawrie, 1966). Whipple et al. (1990) and

Shackelford et al. (1995) identified that differences in beef tenderness are associated with variation in the rate and extent of proteolysis. Proteolysis causes fragmentation of myofibrillar proteins, i.e. protein degradation (Goll et al., 1983). Postmortem aging is a common practice in the beef industry that allows time for proteolysis to occur. Controversy surrounds which proteolytic enzymes drive postmortem protein degradation. Sentandreu et al. (2002) reviewed the identified series of peptidase families that include calpains, cathepsins, proteasomes, caspases, matrix metallopeptidases, and serine peptidases. It was identified that calpains and cathepsins are likely responsible for postmortem protein degradation (Sentandreu et al., 2002).

1.3.1.1 Calpain/Calpastatin system vs. Cathepsins

Calpains are Ca^{2+} dependent proteases (CDP; μ-calpain and m-calpain) that are believed to be responsible for myofibrillar fragmentation that results in postmortem protein degradation (Koohmaraie et al. 1987, 1988, 1990). The calpain system is activated when calcium is released from the mitochondria and sarcoplasmic reticulum postmortem. Calpains are regulated by the specific inhibitor, calpastatin. Koohmaraie et al. (1986) reported that CDPs retain 24 to 28% of activity at a pH of 5.5 to 5.8 and temperature of 5°C. Under these conditions, CDPs are able to reproduce most of the known changes observed in postmortem aging. Furthermore, Koohmaraie et al. (1988) treated slices of the *M. longissimus dorsi* with four solutions varying in chemical composition. Observed effects identified that the only solution containing $CaCl_2$ activated CDP and resulted in a significantly greater amount of myofibrillar

fragmentation and the loss of Z-line(s) structure. However, slices treated with solutions containing metal chelators (ethylene diaminotetraacetic acid [EDTA] or ethylene glycol-bis N,N,N',N'-tetraacetic acid [EGTA]), resulted in the least amount of myofibrillar fragmentation (Koohmaraie et al., 1988). These results support the idea that CDPs are likely responsible for postmortem protein degradation. As previously stated, calpastatin is the regulatory inhibitor of calpains. It is believed that BI cattle have increased calpastatin activity, which decreases calpain activity (Koohmaraie et al., 1990; O'Connor et al., 1997). O'Connor et al. (1997) compared steers and heifers that were 3/8 BI (n=142) to BT steers and heifers (n=115) at a constant fat thickness (11 mm). The calpastatin activity was significantly higher in BI-influenced steers compared to BT steers (4.43 vs. 3.96 units of activity/g). BI-influenced steers and heifers resulted in higher WBS values throughout all postmortem aging day treatments. Pringle et al. (1997) reported similar results. Steers of varying composition (0, 25, 37, 50, 75, and 100% BI influence) were analyzed for calpastatin and μ-calpain activity. Across treatments, calpastatin activity increased while μ-calpain activity decreased as percent BI influence increased. This correlated to increasing WBS values, as percentage of BI increased, at 5 and 10-day postmortem aging (Pringle et al., 1997). Additionally, Wright et al. (2018) reported a decrease in the calpain-calpastatin system correlated to reduced degradation of structural proteins (troponin-T, desmin, and titin). In conclusion, due to increased calpastatin and decreased calpain activity, BI carcasses have higher WBS values and are less tender than BT carcasses.

Cathepsins are proteolytic enzymes that are also thought to aid in postmortem tenderization (Goll et al., 1983). However, while cathepsins have the capacity to produce myofibrillar fragmentation, under purified *in vitro* conditions, degradation of all proteins is observed, even those not normally degraded under postmortem aging conditions. This is because, under normal postmortem conditions in meat, cathepsins are contained in the lysosome. Breakdown of the lysosomal membrane is only observed under high-pressure (40 MPa for 280s, optimal) (Jung et al. 2000). However, when high pressure is applied, cathepsin activity has been shown to decrease (Jung et al. 2000). Additionally, cathepsins are optimally activated when the pH is approximately 4.5 (Poole 1978). This means they are at the optimal activation under conditions inside of the lysosome. When placed in a solution with normal meat pH (5.6 to 5.8), cathepsins are not optimized (Schwartz and Bird 1977). Therefore, cathepsins are not likely responsible for myofibrillar fragmentation and protein degradation postmortem.

1.3.2 Intramuscular and Subcutaneous Fat

Fat deposition amount and distribution differences have been noted between breeds of cattle (Charles and Johnson, 1976). Other factors that influence fat deposition are nutrition, rate of growth, anatomical location of muscle, and muscle fiber type. Of the four fat depots, IMF and SQF are important for sensory attributes and benefit to human health, respectively.

1.3.2.1 Intramuscular Fat

Bos indicus carcasses are generally regarded as lower quality because of reduced ability to deposit IMF. When compared to BT carcasses, numerous studies have reported that BI and BI-influenced cattle have significantly less IMF (Koch et al., 1982; Crouse et al., 1989; Sherbeck et al., 1995). Crouse et al. (1989) reported that as the genetic percentage of BI increases, IMF decreases. However, Crockett et al. (1979) reported that BI crossbreds had more IMF than late-maturing, large-framed BT breeds. Dinh et al. (2010) reported the fatty acid composition of the *M. longissimus dorsi* from purebred BT (Angus) and BI (Brahman) carcasses. BI *M. longissimus dorsi* IMF fatty acid composition was similar (47.77% SFA, 46.9% MUFA, and 5.92% PUFA) compared to BT *M. longissimus dorsi* IMF fatty acid composition (47.83%, 47.81%, and 4.35%, respectively) This indicates that BI IMF contains a higher percentage of PUFA and a lower percentage of MUFA fatty acids in IMF fat compared to BT carcasses.

1.3.2.2 Subcutaneous Fat

Huerta-Leidenz et al. (1993) reported that breed had a significant effect on overall SQF thickness. *Bos indicus* cattle from this study exhibited lower overall body fatness when compared to BT cattle. Subcutaneous fat biopsies were taken at the between the 12th and 13th rib. The fatty acid composition of SQF of BI carcasses was significantly higher in PUFA (2.43%), MUFA (63.43%), and lower in SFA (34.52%) compared to the SQF of BT carcasses (1.83, 59.50, 38.82% respectively) (Huerta-Leidenz et al., 1993). This indicates that BI SQF contains more PUFA and MUFA compared to BT SQF.

Additionally, studies have shown that cuts with increased propensity to deposit fat have an increase in fatty acid composition (Turk and Smith, 2009). Seven fat depots were observed in a study of BT cattle. It was found that the brisket exhibited an elevated percentage of MUFA and PUFA (Turk and Smith, 2009).

1.3.3 Connective Tissue

Connective tissue (CT), as previously discussed, is accountable for part of the variation in tenderness. The three fiber types that compose CT are collagen, elastin, and reticulin. Of these, collagen is the most prevalent. It is understood that structural changes and amount of collagen are affected by animal age (Herring et al., 1967; Norman, 1982). It has been noted that collagen amounts differ between muscles, especially those muscles that are used for locomotion versus support (Ramsbottom et al., 1945). However, research is inconclusive as to whether breed impacts total collagen amount and solubility (Riley et al., 2005). Norman (1982) and Whipple et al. (1990) reported no differences when evaluating total and soluble collagen between BT and BI of animals finished on forage or concentrate diets. However, it was reported that total collagen amount and soluble collagen varied considerably between muscles analyzed within breed (Norman, 1982).

1.3.4 Sensory Comparison

It has been reported that BI meat is less tender than BT meat (Whipple et al., 1990a; Crouse et al., 1993; Pringle et al., 1997; Riley et al., 2005). Protein degradation, or lack thereof, has the largest affect on BI muscle. Due to the inhibition of calpains by

increased calpastatin activity, the result is a decrease in the fragmentation of myofibrils. This, coupled with the fact that BI cattle deposit less intramuscular fat, leads to increased WBS values and less tender cuts than BT cattle (Crouse et al., 1989; Whipple et al., 1990b).

1.4 The Rhomboideus Muscle

The most identifiable characteristic of BI cattle is the "hump," or Rho. The best understanding of the Rho muscle from purebred BI cattle is that it is a phenotypic adaptation for storing lipids. This lipid storage is for animals to draw upon when under environment-related stress is placed on the animal. The Rho muscle accounts for approximately 1.0% of purebred Nellore (BI) chilled carcass weight (Pedrão et al., 2011). In the United States' market, Rho meat is commonly placed in ground beef production. This is due to U.S. consumers not traditionally cooking it as a whole muscle. However, as indicated by Pedrão et al. (2011), in Brazilian markets, the Rho muscle is grilled or barbecued (known as "cupim") and highly sought after. Compositional studies of the Rho muscle from 100% BI genotype cattle have shown increased fat deposition and increased collagen content when compared to that of BT cattle (Pedrão et al., 2009; Pedrão et al., 2011).

1.4.1 Beef muscle profiling

Research has been conducted extensively to profile the muscles present in beef chuck (Calkins, 2001; Von Seggern et al., 2005), where the Rho muscle resides. The

objectives of these studies were to increase the utilization efficiency of beef cuts by identifying muscles that possessed potential to be used as value-added product(s) and/or provide insight for new cuts that could be ascertained through innovative fabrication methods. However, these studies (Calkins, 2001; Von Seggern et al., 2005) assessed BT carcass composition mainly due to geographical location and availability of carcasses when selection and procurement occured. Unique to Von Seggern et al. (2005), is the scale and scope of data collected and analyzed. Twenty-seven muscles from 142 beef chucks were analyzed for proximate composition, pH, total collagen content, Warner-Bratzler shear force (WBS), and objective color. Results from Von Seggern et al. (2005) muscle profiling study for the Rho muscle from BT carcasses were: protein (20.69%), moisture (72.08%), fat (6.35%), pH (5.72), total collagen content (8.49 mg/g), WBS (>4.80 kg), *L** value (41.35), *a** value (28.43), and *b** value (20.99).

Pedrão et al. (2009), although a much smaller study than Von Seggern et al. (2005), examined the Rho muscle and *M. longissimus dorsi* of six purebred Nellore (BI) carcasses raised and finished on native grasses in South America. The proximate composition and collagen content of each was reported. It was found that the Rho has considerably more fat (48.82%), and total collagen (12.40 mg/g) compared to the *longissimus dorsi* (3.39%, 9.56 mg/g, respectively). This indicates that the composition of the Rho muscle from BI carcasses reported by Pedrão et al. (2009) is different than Rho muscles from BT carcasses examined by Von Seggern et al. (2005)

Phenotypic expression (size) of the Rho is the most notable difference between BI and BT cattle. Results from Pedrão et al. (2009) and Von Seggern et al. (2005) suggest vast differences in the composition of the Rho muscle derived from BI or BT carcasses. Additionally Pedrão et al. (2009; 2011), reported that the BI Rho muscle contains an even greater amount of IMF than Japanese Black Waygu *M. longissimus dorsi* (29.7%) (Zembayashi and Nishimura, 1996). However, little research has been conducted to assess the fatty acid composition of the Rho from BI cattle. Turk and Smith (2009), identified that muscles that have increased fat deposition are higher in MUFA and polyunsaturated fatty acids PUFA. These results may also apply to the Rho muscle of BI cattle.

1.5 Summary of Literature Review

Fatty acid composition, collagen content, and calpastatin activity are major causes of flavor development and tenderness, respectively. Little is known about the fatty acid composition of the Rho from BI carcasses. However, previous studies have revealed that the Rho muscle from BI cattle have dramatically increased fat content when compared to the Rho of BT cattle. This indicates that there is potential for the deposition of beneficial fatty acids (MUFA and PUFA). However, the limiting factor affecting consumer acceptability of the Rho muscle from BI carcasses is tenderness. It is known that BI Rho muscle contains more collagen and has higher calpastatin activity (limiting proteolysis of myofibrillar proteins postmortem) when compared to BT Rho (Von Seggern et al., 2005; Pedrão et al., 2009). It is inferred that the Rho muscle derived from BI cattle

should result in increased WBS values and be less tender than the Rho muscle from BT cattle.

1.6 Project Objectives

The hypothesis of this study was that increased fat deposition in the Rho would lead to an increase in the percentage of MUFA. The primary objective of this study was to assess the fatty acid composition of the lean portion and SQF depot of the Rho from BI-influenced cattle. Additional objectives were to identify and quantify the proximate composition, collagen content, and sensory attributes of the Rho from BI-influenced cattle.

CHAPTER II

MATERIAL AND METHODS

2.1 Animals and facilities

M. Rhomboideus muscles were procured from a commercial beef harvest and processing facility in Texas and carcasses were harvested under FSIS directive 6900.2. This research was approved by Texas A&M University IRB (IRB2018-1077M). Institutional Animal Care and Use Committee approval for this study was not required; all harvest activities were conducted by employees of the commercial harvest establishment. Cattle were slaughtered, weighed (hot carcass weight, HCW) and held for two days postmortem (<4.4°C). Carcasses were then separated, between the 12th and 13th rib, and the *M. longissimus thoracis* was exposed to oxygen ("bloom") for 15 minutes. Carcasses were assigned quality grades by a USDA grader. Hot carcass weight, *M. longissimus thoracis* area, and external fat thickness data (measured at 3/4 of the length of the ribeye from the split chine bone) were collected.

2.2 Selection Criteria

Three replications of 4 USDA Choice (Small 00 to Moderate 99) and 4 USDA select (Slight 00 to Slight 99) Rho muscles were selected from the right half of split beef carcasses (n = 24). *M. Rhomboideus* selection parameters were >7.62 cm hump height, >7.62 cm width, >25.4 cm length, weight range: 2 to 4 kg.

2.3 Sample Collection and Preparation

M. Rhomboideus muscles were removed prior to carcass fabrication. The dorsal portion of the Rho muscle was removed in a manner similar to that described for the purchaser specified option 4 of the Institution Meat purchase specifications 116A, beef chuck, chuck roll (North American Meat Insititue, 2014). The exception to this cutting style being that the dorsal edge of the Rho was not trimmed to be parallel to the arm (ventral) edge of the chuck roll. Muscles were individually vacuum-packaged and transported with ice in an insulated cooler packed in ice to the Texas A&M University Rosenthal Meat Science and Technology Center (College Station, Texas). Muscles were allowed to wet-age an additional 12 d in refrigerated storage (< 4.4° C). Muscles were then placed in a freezer for 12 h (< -10° C). From each end of each Rho muscle 3.81 cm steaks were removed using a Marel bandsaw (Marel, Garðabæ, Iceland). Steaks were vacuum-packaged and utilized to determine proximate composition. This prepared a flat cutting edge that 2.54 cm serial steaks could be fabricated (anterior to posterior) from the remaining Rho muscle. Steaks were individually labeled, vacuum-packaged, and stored under freezer conditions (< -10° C). Before each analysis, steaks were removed from the freezer and allowed to temper (12 h; <4.4° C). Serial steak designation for all analyses is depicted in **Figure 1**.

2.4 Proximate Composition

The 1st and 10th serially cut steaks from each Rho muscles were used to determine proximate composition (Figure 1). Steaks were diced into approximately 0.64 cm cubes

and flash-frozen in liquid nitrogen. Cubes produced from steaks 1 and 10 were powdered together using a Waring blender (Model 33BL79, Waring Commercial, New Hartford, CT). Metal powdering canisters were washed and dried between each sample. Powered samples were collected in labeled bags and stored in Fisher-Scientific Isotemp freezer (-80° C, Fisher-Scientific, Pittsburgh, PA) until analyzed. Powdered samples were used to determine proximate composition [AOAC, 1990; 2005, 950.46 (moisture), 960.39 (fat), 992.15 (protein)].

2.4.1 Moisture

procedure 950.46 was used to calculate moisture. Thimbles were constructed from folded Whatman #1 filter paper (125 mm, GE Healthcare, Chicago, IL) filter paper that was formed into a sleeve with one end open and the other end stapled. Thimbles were labeled with a #2 pencil and dried for a minimum of 12 h at 100° C in an VWR gravity convection oven (VWR 1350GM, VWR International, Radnor, PA) equipped with desiccant. Dried thimbles were then removed and placed in a desiccator and allowed to cool for 30 min. Thimbles were weighed and their weights recorded individually to the nearest 0.0001 g (initial thimble weight, A). Two to three grams of powdered sample were added to thimble and weight recorded to the nearest 0.0001 g (wet sample + thimble weight, B). Each sample was weighed in triplicate. The open end of the thimble was folded over and stapled. This weight was recorded (wet sample + thimble + staple, C) The thimbles were returned to the oven and dried at 100° C for 16 to 18 h. Thimbles were removed and allowed to cool in a desiccator for 1 h. Dried samples were weighed

to the nearest 0.0001 g and weights recorded (dried sample + thimble + staple, D). Percent moisture was determined: [(C - D)/(B - A)] x 100.

2.4.2 Fat

Association of Analytical Chemists (1990b) procedure 960.39 was used to calculate fat. Using dried samples from moisture determination, 10 to 12 dried samples were placed in a Soxhlet apparatus for ether extraction to determine fat content. Parafilm was used to seal the apparatus at junction points. Petroleum ether was boiled and allowed to condense into the apparatus at a rate of 4 to 6 drops/second. Samples were extracted for 18 h continuously. Heating plates were shut off and remaining evaporated ether was allowed to condense for 30 min post-shutdown. Extracted thimble samples were removed from the Soxhlet apparatus and placed into desiccator for 30 min. Extracted samples were placed in an air-dry oven set at 100° C for 12 h. Dried extracted samples were removed from the oven and allowed to cool for 30 min in a desiccator before weighing and recording the thimble weight to the nearest 0.0001 g (dried extracted sample plus thimble and staple, E). Percent fat was determined: [(D - E)/(B - A)] x 100.

2.4.3 Protein

Association of Analytical Chemists (2005) procedure 992.15 was used to calculate protein. Approximately 0.5 g of powdered sample was weighed into Leco tin foil cups (Leco Corporation, St. Joseph, MI). Exact weights were recorded. Samples were analyzed using Leco FP-528 nitrogen analyzer (FP-528, Leco Corporation, St. Joseph, MI). Nitrogen content was multiplied by 6.25 factor to produce protein percentage.

2.5 Fatty Acid Profile

From each Rho steak the subcutaneous fat was removed and analyzed for fatty acid composition separately. The remaining steak muscle (lean portion) and subcutaneous fat were diced into approximately 0.64 cm cubes and reduced in size using a Cuisinart 14-cup food processor (Cuisinart, Stamford CT). The food processor was rinsed and dried between each sample.

2.5.1 Extraction of Total Lipids

Duplicate samples (1 to 5 g) were measured and added to a labeled 50 mL glass tube. Fatty acids were extracted using the modified version of Folch et al. (1957). Five milliliters of chloroform:methanol (CHCl3:CH3OH) at a 2:1 ratio were added to tubes and the samples were homogenized using a Polytron homogenizer (Kinematica Polytron PT 10/35GT Homogenizer, Fisher Scientific, Pittsburgh, PA) on medium setting (7000 to 8000 rpm) for 30 s. After homogenization, the probe was rinsed with CHCl3:CH3OH and collected into 50 mL test tube to produce a final volume of 15 mL. The samples were allowed to sit for 30 min at room temperature to extract lipids. Homogenate was filtered through a Whatman #1 filter paper (125 mm, GE Healthcare, Chicago, IL), into a second labeled 50 mL centrifuge tube. The first tube was rinsed 2 to 3 times with 2 mL of CHCl3:CH3OH. Eight milliliters of 74% potassium chloride (KCl) was added to each test tube and were vortexed for 1 min. The solution was allowed to sit and separate at room temperature for 2 h. The lower phase was transferred to a labeled 20-mL

scintillation vial. The scintillation vial was evaporated to dryness with nitrogen using an evaporator (N-Evap 112, Organomation, Berlin, MA) with the water bath set at 40° C.

2.5.2 Saponification and Methylation of Lipids

One milliliter of 0.5 N KOH in MeOH were added to the scintillation vial. The vial was heated in a 70° C water bath for 10 min. One milliliter of 14% boron trifluoride (BF_3) in, suspended in MeOH, was then added and the tube flushed with N_2 and tightly capped. The scintillation vial was returned to the 70° C water bath for 30 min. Following the heating cycle, the vial was allowed to cool for 10 min. This procedure saponified the lipids, i.e., it liberated the fatty acids from the glycerol backbone. The fatty acid was methylated in the process, removing the net negative charge of this group. Two milliliters of HPLC-grade hexane were added with 2 mL of saturated NaCl and vortexed for one minute. The upper layer of the solution was pipetted off and placed in a labeled 20 mL glass scintillation vial containing 800 mg of NaSO4, to remove any excess moisture. Two milliliters of hexane were added to the tube and vortexed. The upper layer was pipetted off into a labeled 20 mL test tube. This process of adding hexane and pipetting off the upper layer was repeated in triplicate. The samples were evaporated (N-Evap 112, Organomation, Berlin, MA) with the water bath set at 40° C. The sample were reconstituted with the appropriate amount of hexane to obtain approximately 50 mg/mL. Four hundred microliters of this solution were pipetted into a 2 mL autosampler vial containing 1.6 mL of HPLC grade hexane. The autosampler vials were analyzed using gas chromatography to detect the presence of fatty acids.

2.6 Collagen Content

2.6.1 Separation of Soluble and Insoluble Collagen

Rho steaks were powdered and stored in the same manner as described in section 2.4. Duplicate samples from each powdered steak were weighed out (3 to 4 g) and exact weight was recorded. The samples were placed in 50-mL disposable centrifuge test tubes. To each tube 12 mL of one-quarter strength Ringer's solution were added. Each tube was then stirred exactly 10 times, capped, and placed into a water bath (50° C) for 15 min. The tubes were allowed to cool in a 4.4° C cooler for 15 min. Tubes were centrifuged for 20 min at *21,525 x g* at 2° C using a JA-17 rotor (Beckman Coulter, Fullerton, CA). The supernatant was decanted into a cooking jar (6.35 cm x 6.95 cm). Eight milliliters of 1/4 strength Ringer's solution was added to the test tube containing the residual pellet and stirred ten additional times, using a clean glass rod for each sample. Tubes were again heated, cooled, centrifuged, and decanted into the same cooking jar. Five milliliters of ddH_2O were added to the residual meat pellet and mixed well. The pellet and water were poured into a separate cooking jar. Tubes were wiped clean and any residue was added to the residual pellet. Four additional milliliters of ddH_2O were added to the residual pellet. Twenty-five milliliters of concentrated HCl (12 N) were added to each supernatant and 25 mL of 6N HCl were added to residual pellet. All cooking jars were sealed with a Teflon gasket and capped and placed in an oven under a fume hood, where they were cooked at 105 °C for 16 h. When the 16 h had elapsed, the supernatant and residual pellets were removed from the oven and lids were

immediately removed. Both the supernatant and residual pellet were allowed to cool under a fume hood (1 h), allowing vapors to escape. After cooling, the supernatants were transferred to 200 mL volumetric flasks and the residual pellets were transferred to 500 mL volumetric flasks respectively. All cooking jars were rinsed with 25 mL of ddH_2O and added to their respective volumetric flasks. The volumetric flasks were then be brought to volume with ddH_2O. Each sample were filtered through Whatman #2 filter paper (125 mm, GE Healthcare, Chicago, IL) into 50 mL test tubes, collecting at least 50 mL. The remaining liquid was discarded.

2.6.2 Hydroxyproline Standard Curve

A hydroxyproline stock solution was made using trans-4-hyrdroxy-L-proline (Sigma-Aldrich, St. Louis, MO) by dissolving 0.025 g of hydroxyproline powder into 250 mL of 0.001 N HCL. Five 100 mL standard solutions were made by adding hydroxyproline stock solution (2, 4, 6, 8, and 10 mL) to ddH_2O (98, 96, 94, 92, and 90 mL). This formed the standardized color curve.

2.6.3 Color Development and Absorbance

Two milliliters of each standard solution were added to duplicate 20 mL test tubes, along with two blank 20 mL tubes with 2 mL of ddH_2O. Two milliliters of each supernatant and residual pellet were added to individual 20 mL test tubes. To all tubes, including standards and blanks, 1 mL was added of oxidant solution (1.41 g chloramine T, Fisher Scientific, Pittsburgh, PA) dissolved in 100 mL of buffer solution, 30.0 g citric acid monohydrate, 15.0 g NaOH, 90 g sodium acetate trihydrate, dissolved in 500 ml

ddH_2O then put in a 1 L volumetric flask to which 290 mL isopropanol was added). The tubes were vortexed for 30 min and then rested for exactly 20 min at room temperature. One milliliter of color reagent was added (10 g of 4-dimethybenzaldehyde, Fisher Scientific, Pittsburgh, PA) in 30 mL of 70% perchloric acid to which 65 mL of isopropanol was slowly added). All 20 mL tubes were vortexed, covered with aluminum foil, and placed in a 60° C water bath for 15 min. After the 15 min, the tubes were removed, uncovered and allowed to cool to room temperature. Once cooled, 250 μL of each sample solution was transferred to a 96-well microplate and read using an Epoch microplate spectrophotometer (BioTek, Winooski, VT) at 558 nm absorbance. The blanks and standards were read first and the readings recorded. Using Microsoft Excel 2016 (Microsoft Corp., Redman, WA), the readings were fit to the standard curve to determine the milligrams of hydroxyproline in each sample. The hydroxyproline amount was multiplied by the dilution factors (200 for the supernatant and 500 for the residual), and divided by the initial sample weight of each individual sample, this produced the total hydroxyproline amount (mg). To determine the actual collagen amount the hydroxyproline amount was multiplied by 7.52 for the supernatant and 7.25 for the residual (Cross et al., 1973) and then divided by 100 to give the supernatant and residual collagen amounts in mg/g. These methods are derived from Eilert and Mandigo (1993) who derived their methods from Woessner (1962), Hill (1966), and Cross et al. (1973).

2.7 Color Determination

Rho steaks were removed from vacuum packaging and exposed to oxygen for 15 min at room temperature. Raw color measurements (*L**, *a**, *b**) were taken using a HunterLab Miniscan XE plus (3.18 cm aperture and 10° standard observers; Hunter Associates Laboratory, Inc., Reston, VA) from the center of each steak. Spectrophotometer was standardized using white and black standard tiles. Triplicate readings were taken from the geometric center of each steak.

2.8 pH Assessment

Ten grams of powdered sample acquired from the 4th steak of each Rho muscle were blended with 90 mL of ddH_2O for 30 s. pH was determined using a benchtop pH meter (VWR Symphony Red Tip Reference Probe, VWR International Radnor, PA). Triplicate readings were taken from each solution.

2.9 Cooking, Sensory Analysis, and Warner Bratzler Shear Force

2.9.1 Cooking

Rho steaks were cooked on a 2.54 cm thick, flat top Star Max 536TGF 91.44 cm Countertop Electric Griddle with Snap Action Thermostatic Controls (Star International Holdings Inc. Company, St. Louis, MO) set at 180° C ± 2.8° C. Steaks had an copper-constantan thermocouple (Omega Engineering, Stanford, CT) inserted into the geometric center to monitor internal steak temperature using an Omega HH501BT Type-T thermometer (Omega Engineering, Stanford, CT). Steaks were turned at an

internal temperature of 35° C and removed at 71° C (medium degree of doneness). Grill temperature was monitored by randomly selecting locations on the griddle top using a handheld instantaneous surface thermometer (Pro-Surface ThermaPen, ThermoWorks, American Fork, UT).

2.9.2 Trained Sensory Panel

A beef flavor descriptive attribute panel (n = 5) with over 200 h of training and 26 y of experience were trained to assess 10 basic flavors, 31 secondary flavors, and 2 texture attributes from the beef lexicon (Adhikari et al., 2011). Panelists were trained to scale each attribute on a 16-point intensity scale (0 = none and 16 = extremely intense). Each day, panelists were served two "warm-up" samples, which were discussed verbally to insure proper scaling and precision of scoring. Panelists were served two random and representative cubes (1.3 cm x 1.3 cm x steak thickness), in a plastic soufflé cup. Samples were served through a breadbox style booth under red lighting. Saltless crackers and double distilled were offered as palette cleansers. Twelve samples were tested each day, with a minimum of 4 min between each sample with a break after the sixth sample.

2.9.3 Warner-Bratzler Shear Force

Rho steaks used for WBS were tempered and cooked in the same manner as described in section 2.9.1. Post-cooking steaks were stored over night at 4.4° C. Up to six cores, 1.3 cm in diameter, were removed parallel to the muscle fibers. Each core was sheared once, perpendicular to the muscle fibers, on a United Testing machine (United

SSTM-500, Huntington Beach, CA). The crosshead speed was 200 mm/min using a 500 kg load cell, and a 1.02 cm thick V-shape blade with a 60° angle. The peak force (kg) needed to shear each core was recorded to determine tenderness.

2.10 Statistical Analysis

Data were analyzed using one-way ANOVA with JMP Pro v13.0.0 (SAS Institute, Inc., Cary, NC) with quality grade (USDA Choice and USDA Select) as the main effects. Sensory data were analyzed using a linear fit model with order as a random effect. Least squares means were calculated for significant main effects. Significant differences among means were determined using Tukey's HSD with $P<0.05$.

CHAPTER III

RESULTS SUMMARY AND DISCUSSION

3.1 Carcass Characteristics

Differences in carcass characteristic data of selected carcasses were only present for quality grade ($P < 0.001$; Table 1). These results were expected as quality grade was the main effect for this study. No statistical differences ($P > 0.05$) were found between treatments for hot carcass weight, ribeye area, or fat thickness.

3.2 Proximate Composition and Collagen Content

3.2.1 Proximate Composition

No statistical differences were found between Ch and Se for percent moisture ($P = 0.25$; Table 2) or percent fat ($P = 0.21$; Table 2) of the Rho. Between Ch and Se treatments in this study, percent protein of Rho muscles was higher ($P = 0.019$; Table 2) for Ch (19.36%) compared to Se (18.58%). Mean percent moisture (68.27%) for this study was much higher than values reported by Pedrão et al. (2009) for Rho muscles from purebred Nellore (BI) carcasses (36.70%). Additionally, mean percent fat (11.28%) in this study was much lower than the value (48.82%) stated by Pedrão et al. (2009). Numerous studies suggest that concentrate-based diets increase IMF (Suzuki et al., 1976; Rule et al., 1987; Mandell et al., 1998). However, Naik (1978) described the Rho muscle as an energy reservoir of fats and fatty acids during events of stress. This could mean that under natural production systems, as described by Pedrão et al. (2009), the Rho muscle's

propensity to deposit intramuscular could be higher. Finally, mean percent protein (18.97%) was slightly higher than percent protein presented by Pedrão et al. (2009) (12.60%). Proximate composition percentages in this study were more similar to BT Rho muscles (72.08% moisture, 6.35% fat, 20.69% protein) reported by Von Seggern et al. (2005). This could indicate that carcasses used in this study were greater influenced by BT genetics. It has been suggested by Sherbeck et al. (1996) that genotype and Rho muscle height expression has a moderate correlation ($r^2 = 0.59$). However, Sherbeck et al. (1996) concluded that cattle with the same BI genetic percentages could differ in phenotypic expression of BI breed characteristics. Sherbeck et al. (1996) reported that 22% cattle from a study of 160 steers with known 25% or less BI genetics had a hump height that was greater than 7.62 cm. The selection parameter for Rho height in this study was >7.62 cm, and Rho muscles potentially could have been harvested from cattle possessing a lower degree of BI genetics, thus affecting the results reported in this study.

3.2.2 Collagen Content

Total collagen content, insoluble collagen, and soluble collagen content was higher ($P < 0.001$; Table 2) for Ch Rho muscles (21.92, 21.56, 0.26 mg/g, respectively) compared to Se Rho muscles (13.87, 13.73, 0.14 mg/g, respectively). Legako et al. (2015) and Li et al. (2006) reported a general trend that as quality grade increases, collagen content also increases. This is primarily due to adipose tissue being deposited in connective tissue. This means a greater amount of intramuscular fat will require a greater amount of collagen for storage. The results of the present study for total collagen were similar to the reported value reported by Pedrão et al. (2009) (12.40 mg/g). The collagen content

of the Rho muscle is much higher than other BT muscles (Hill, 1966; Herring et al., 1967; Genho, 2009). This is due to the function of the Rho muscle, as it is thought to aid in locomotion (Pedrão et al., 2012). Numerous others agree (Ramsbottom et al., 1945; Norman, 1982; Von Seggern et al., 2005) that collagen content is often dependent on the muscle type and function. Interestingly, in the present study, a relatively low percentage (< 2%) of soluble collagen was present in the Rho muscles for both quality grades compared to Hill (1966), Herring et al. (1967), and Genho (2009). Future research should focus on identifying the reason for the low percentage of soluble collagen.

3.3 Fatty Acid Composition (FAC)

3.3.1 Lean Portion

Total MUFA, PUFA, SFA percentages, and MUFA:SFA ratio of the lean portion of Rho muscles were not different ($P > 0.05$; Table 3) between Ch muscles compared to Se muscles. Means across both quality grades for the lean portion of Rho muscles for total MUFA (42.22%), PUFA (6.76%), and SFA (46.07%) were similar to reported fatty acid composition of purebred BT *longissimus dorsi* lean portion (47.81, 4.35, 47.83%, 1.0, respectively) (Dinh et al., 2010). There is a slight increase in PUFA percentages present in the lean portion of the Rho muscle. Bressan et al. (2011) described a lower rate of ruminal biohydrogenation of fatty acids in BI cattle. This indicates there is a higher chance for PUFA passage through the rumen and incorporation longer unsaturated chain fatty acids to be deposited in fat of BI cattle. Thus, this could support the theory that the Rho is used for essential fatty acid storage and utilization during periods of stress, such

as drought (Naik, 1978). Statistical differences ($P < 0.05$) were observed between quality grades for individual MUFA, PUFA, and SFA. However, when assessed on a numerical basis the fatty acids that are statistically different account for a small percentage of the fatty acid composition of the Rho muscle.

3.3.2 Subcutaneous Fat (SQF)

Total MUFA percentage was significantly higher ($P < 0.001$) in Ch Rho SQF compared to Se Rho SQF. Additionally, total PUFA and SFA percentage were higher ($P < 0.02$; Table 4) in Se Rho SQF compared to Ch Rho SQF. Huerta-Leidenz et al. (1993) stated that SQF of the *longissimus dorsi* of BI cattle contains more MUFA and PUFA than the SQF of the *longissimus dorsi* of BT cattle. Means across both quality grades of Rho muscle SQF for total MUFA (47.38%), PUFA (4.00%), and SFA (48.61%) were compared to reported FAC averages of BT brisket SQF (56.58, 2.53, and 35.87%; respectively). The brisket is a fat depot known for elevated MUFAs (Turk and Smith, 2009). This indicates that the BI-influenced SQF in the present study was numerically lower than brisket SQF. However, Pedrão et al. (2012) reported total MUFA (56.67%) and PUFA (2.77%) percentages from purebred Nellore (BI) Rho muscles that were more comparable to brisket SQF described Turk and Smith (2009). This difference between purebred Nellore and BI-influence Rho muscle could be described differences in nutrition provided. Naik (1978) hypothesized that Rho muscle deposited fat and energy on natural production systems, such as those described by Pedrão et al. (2012).

Nevertheless, in the present study, total fatty acid percentages were lower than previously reported data of Rho muscles from BI carcasses.

3.4 Sensory Descriptive Attribute and Warner-Bratzler Shear Force Values

No statistical differences ($P > 0.05$; Table 6) were observed for any sensory characteristics between Ch and Se Rho muscles. However, WBS values were significantly greater ($P < 0.001$; Table 5) for Se (3.15 kg) compared to Ch (2.58 kg). This result was not expected as Ch Rho muscles contain 1.5-times as much collagen as Se Rho muscles. A possible cause of the difference in WBS between Ch and Se Rho muscles is the fat content of the muscles. Aberle et al. (2012) described that muscles with a greater IMF are perceived as more tender because of the fats lower pressure resistance and a disruption in connective tissue by the adipocytes (fat) deposition. Nevertheless, Pedrão et al. (2009) concluded the same results in Rho muscles of purebred Nellore (BI) carcasses. Due to the increased IMF deposition in the Rho muscle WBS values were reduced. A muscle with a WBS value of < 3.9 kg has a 68% chance of being rated slightly tender or higher (Shackelford et al., 1991). Average of the means across both quality grades for WBS values in this study was 2.87. This indicates that the Rho muscle has a high chance of being rated as tender and has merit to be accepted by consumers. The results of this study were confirmed by trained sensory panelists, who determined no significant differences ($P > 0.05$; Table 5) existed for muscle fiber tenderness or connective tissue between quality grades.

3.5 Conclusions

The objective to assess the fatty acid composition FAC of the IMF and SQF depots in the Rho muscles from BI crossed cattle was achieved. The fatty acid composition of the Rho was found to be unique as compared to the findings Turk and Smith (2009) and Pedrão et al. (2012). Although it was not observed that there were elevated MUFA present in the Rho as hypothesized, it was concluded that the percentage of PUFAs in SQF of Rho muscles was higher than that of BT brisket SQF and purebred Nellore (BI) Rho SQF. Additionally, the percentage of PUFAs in IMF of Rho muscles from the present study were found to be elevated when compared to BT *longissimus dorsi* IMF. This indicates that the Rho muscle has some value as a source of PUFA. It has been discussed that PUFA benefit human health by reducing cardiovascular disease.

Additional objectives were to identify and quantify the proximate composition, collagen content, and sensory attributes of the Rho from BI crossbred cattle. These objectives were accomplished. It was revealed that Rho muscle was tender as determined by WBS values. These result shows that BI meat is not always tough as indicated by numerous studies. Future research is needed to address proper preparation and cookery methods for the Rho muscle. However, the Rho muscle is potentially a cut that could offer economic gain with proper marketing.

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APPENDIX A

TABLES

Table 1. Least squares means of carcass data from USDA Choice and USDA Select *Bos indicus* carcasses

	Treatment			
Variable	USDA Choice[1]	USDA Select[2]	SEM[3]	*P*-value[4]
n	12	12		
Carcass Data				
HCW[5], kg	384	384	13.9	0.99
USDA Quality Grade[1,2]	477.5[a]	360.0[b]	11.15	0.0001
REA[6], sq cm	89.52	89.30	2.848	0.96
Fat Thickness[7], cm	1.57	1.54	0.029	0.91

[1]USDA Choice, marbling score Small[00] to Small[99] (400 to 499), Modest[00] to Modest[99] (500 to 599), Moderate[00] to Moderate[99] (600 to 699),
[2]USDA Select, marbling score: Slight[00] to Slight[99] (300 to 399)
[3]SEM: standard error of the mean (largest) of the least squares means
[4]*P*-values < 0.05 are considered significant
[5]HCW: Hot carcass weight
[6]REA: Ribeye Area, taken at $12^{th}/13^{th}$ rib interface
[7]Fat thickness taken at the $12^{th}/13^{th}$ rib interface, 3/4 length of the ribeye

Table 2. Least squares means for proximate composition, collagen content, color measurements, and pH between USDA Choice and USDA Select steaks from the *M. Rhomboideus* of *Bos indicus* carcasses

	Treatment			
Variable	USDA Choice[1]	USDA Select[2]	SEM[3]	*P*-value[4]
n	12	12		
Proximate Composition				
Protein, %	19.36[a]	18.58[b]	0.015	0.019
Moisture, %	67.81	68.73	0.574	0.25
Fat, %	11.90	10.66	0.701	0.21
Collagen Content				
Total Collagen	21.92[a]	13.87[b]	1.004	0.001
Insoluble Collagen	21.56[a]	13.73[b]	0.998	0.001
Soluble Collagen, mg/g	0.26[a]	0.14[b]	0.020	0.0001
Soluble Collagen, %	1.21	1.04	0.120	0.339
Color Measurements				
Lightness, L*	47.95[b]	50.27[a]	0.773	0.0368
Redness, a*	15.72	15.34	0.492	0.58
Yellowness, b*	13.54	13.56	0.366	0.97
pH	5.66	5.64	0.015	0.43

[1]USDA Choice, marbling score Small[00] to Small[99] (400 to 499), Modest[00] to Modest[99] (500 to 599), Moderate[00] to Moderate[99] (600 to 699),
[2]USDA Select, marbling score: Slight[00] to Slight[99] (300 to 399)
[3]SEM: Standard error of the mean (largest) of the least squares means
[4]*P*-values < 0.05 are considered significant
mean (largest) of the least squares means
[3]*P*-values < 0.05 are considered significant
[ab]LSMeans within a row that lack a common superscript differ ($P < 0.05$)

Table 3. Least squares means of the fatty acid composition between USDA Choice and USDA Select intramuscular fat from the *M. Rhomobidoeus* of *Bos indicus* carcasses

	Treatment			
Variable	USDA Choice[1]	USDA Select[2]	SEM[3]	*P*-value[4]
n	12	12		
Fatty acids, %[5]				
C12:0	0.10	0.08	0.011	0.41
C14:0	4.63[a]	4.15[b]	0.132	0.0128
C16:0	30.01	29.31	0.437	0.26
C18:0	14.21[b]	16.73[a]	0.495	0.0007
C20:0	0.17[b]	0.32[a]	0.035	0.0056
C22:0	0.02	0.01	0.008	0.68
C24:0	0.12	0.09	0.012	0.08
C:14:1	1.08[a]	0.86[b]	0.045	0.0012
C16:1	3.66[a]	2.91[b]	0.148	0.0007
C18:1 cis-9	33.91	35.34	0.725	0.17
C18:1 cis-11	4.17	3.48	0.250	0.54
C20:1	0.25	0.35	0.027	0.99
C22:1	0.02	0.01	0.008	0.67
C24:1	0.19[a]	0.12[b]	0.021	0.0267
C18:2	6.02	5.38	0.335	0.18
C18:3	0.15	0.17	0.015	0.44
C20:2	0.24[a]	0.12[b]	0.025	0.0019
C20:3	nd	nd	nd	nd
C20:4	1.07[a]	0.68[b]	0.121	0.024
C20:5	nd	nd	nd	nd
C22:6	0.00	0.00	0.000	0.33
Total MUFA[6]	41.47	42.97	1.494	0.48
Total SFA[7]	47.19	50.69	1.637	0.14
MUFA:SFA[8]	0.89	0.86	0.029	0.47
Total PUFA[9]	7.17	6.34	0.493	0.24

[1]USDA Choice, marbling score Small[00] to Small[99] (400 to 499), Modest[00] to Modest[99] (500 to 599), Moderate[00] to Moderate[99] (600 to 699),

[2]USDA Select, marbling score: Slight[00] to Slight[99] (300 to 399)

[3]SEM: Standard error of the mean (largest) of the least squares means

[4]*P*-values < 0.05 are considered significant

[5]nd: not detected

[6]Total monounsaturated = [14:1 + 16:1 + 18:1C9 + 18:1C11 + 20:1 + 22:1 + 24:1]

[7]Total saturated = [12:0 + 14:0 + 16:0 + 18:0 + 20:0 + 24:0]

[8]Monounsaturated:saturated fatty acid ratio = $\frac{\text{Total MUFA}}{\text{Total SFA}}$

[9]Total polyunsaturated = [18:2 + 18:3 + 20:2 + 20:3 + 20:4 + 20:5 + 22:6]

[ab]LSMeans within a row that lack a common superscript differ (P < 0.05)

Table 4. Least squares means of the fatty acid composition between USDA Choice and USDA Select subcutaneous fat from the *M. Rhomboideus* of *Bos indicus* carcasses

	Treatment			
Variable	USDA Choice[1]	USDA Select[2]	SEM[3]	*P*-value[4]
n	12	12		
Fatty acids, %[5]				
C12:0	0.09	0.07	0.010	0.40
C14:0	4.47	4.33	0.117	0.38
C16:0	29.49	30.18	0.386	0.21
C18:0	12.41[b]	15.20[a]	0.536	0.0006
C20:0	0.49	0.48	0.059	0.88
C22:0	nd	nd	nd	nd
C24:0	0.00	0.00	0.001	0.31
C:14:1	1.31[a]	0.99[b]	0.060	0.0005
C16:1	4.01[a]	3.26[b]	0.214	0.0161
C18:1 cis-9	39.40[a]	37.08[b]	0.516	0.0024
C18:1 cis-11	4.35	3.80	0.328	0.24
C20:1	0.25	0.25	0.028	1.00
C22:1	nd	nd	nd	nd
C24:1	0.00	0.00	0.001	0.31
C18:2	3.59[b]	4.16[a]	0.162	0.0156
C18:3	0.09	0.14	0.020	0.06
C20:2	0.00	0.01	0.004	0.18
C20:3	nd	nd	nd	nd
C20:4	0.01	0.01	0.006	0.67
C20:5	nd	nd	nd	nd
C22:6	nd	nd	nd	nd
Total MUFA[6]	49.35[a]	45.41[b]	0.699	0.0002
Total SFA[7]	46.95[b]	50.27[a]	0.700	0.0015
MUFA:SFA[8]	1.07[a]	0.92[b]	0.029	0.0005
Total PUFA[9]	3.69[b]	4.32[a]	0.168	0.0105

[1]USDA Choice, marbling score Small[00] to Small[99] (400 to 499), Modest[00] to Modest[99] (500 to 599), Moderate[00] to Moderate[99] (600 to 699),

[2]USDA Select, marbling score: Slight[00] to Slight[99] (300 to 399)

[3]SEM: Standard error of the mean (largest) of the least squares means

[4]*P*-values < 0.05 are considered significant

[5]nd: not detected

[6]Total monounsaturated = [14:1 + 16:1 + 18:1C9 + 18:1C11 + 20:1 + 22:1 + 24:1]

[7]Total saturated = [12:0 + 14:0 + 16:0 + 18:0 + 20:0 + 24:0]

[8]Monounsaturated:saturated fatty acid ratio = $\frac{\text{Total MUFA}}{\text{Total SFA}}$

[9]Total polyunsaturated = [18:2 + 18:3 + 20:2 + 20:3 + 20:4 + 20:5 + 22:6]

[ab]LSMeans within a row that lack a common superscript differ (P < 0.05)

Table 5. Least squares means of trained sensory scores for beef identity, juiciness, muscle fiber tenderness, and connective tissue and Warner-Bratzler shear force values between USDA Choice and USDA Select from the *M. Rhomboideus* of *Bos indicus* carcasses

	Treatment			
Variable	USDA Choic[1]	USDA Select[2]	SEM[3]	*P*-value[4]
n	12	12		
Palatability Trait[5]				
Beef ID	7.68	7.79	0.139	0.61
Juiciness	8.72	9.05	0.181	0.27
Muscle Fiber Tenderness	7.29	7.52	0.394	0.95
Connective Tissue	4.79	5.15	0.00	0.75
WBS[6], kg	2.58[b]	3.15[a]	0.102	0.0001

[1]USDA Choice, marbling score Small[00] to Small[99] (400 to 499), Modest[00] to Modest[99] (500 to 599), Moderate[00] to Moderate[99] (600 to 699),
[2]USDA Select, marbling score: Slight[00] to Slight[99] (300 to 399)
[3]SEM: Standard error of the mean (largest) of the least squares means
[4]*P*-values < 0.05 are considered significant
[5]Palatability traits were reported on a 16-point scale (0 – no detection to 16 – very intense/strong)
[6]WBS: Warner-Bratzler shear force
[ab]LSMeans within a row that lack a common superscript differ ($P < 0.05$)

Table 6. Least squares means of trained sensory scores for palatability traits between USDA Choice and USDA Select of steaks from the *M. Rhomboideus* of *Bos indicus* carcasses

Variable	Treatment		SEM[3]	*P*-value[4]
	USDA Choice[1]	USDA Select[2]		
n	12	12		
Palatability Trait[5]				
Brown	8.09	8.20	0.225	0.72
Roasted	6.40	6.43	0.128	0.88
Bloody-Serumy	1.67	1.66	0.104	0.97
Fat-Like	3.89	4.48	0.267	0.13
Bitter	2.21	2.06	0.060	0.12
Salty	1.88	1.96	0.059	0.36
Sweet	1.48	1.71	0.124	0.16
Sour	2.19	2.10	0.073	0.45
Umami	3.97	4.23	0.149	0.28
Metallic	2.29	2.17	0.076	0.20
Overall Sweet	0.65	0.73	0.103	0.59
Animal Hair	nd	nd	nd	nd
Asparagus	0.02	0.00	0.015	0.32
Barnyard	0.02	0.00	0.015	0.32
Beet	nd	nd	nd	nd
Burnt	0.02	0.00	0.015	0.32
Buttery	0.68	0.99	0.164	0.19
Cardboardy	1.50	1.37	0.155	0.56
Chemical	0.00	0.02	0.015	0.32
Cocoa	nd	nd	nd	nd
Cooked Milk	0.00	0.02	0.015	0.32
Cumin	nd	nd	nd	nd
Dairy	nd	nd	nd	nd
Green	0.04	0.00	0.031	0.32
Green Hay	0.00	0.02	0.015	0.32
Heated Oil	0.04	0.08	0.034	0.40
Leather	0.02	0.00	0.015	0.32
Liver-Like	0.00	0.02	0.015	0.32
Musty Earthy	1.13	1.15	0.131	0.92
Refrigerator Stale	nd	nd	nd	nd
Rancid	nd	nd	nd	nd
Smokey Charcoal	0.041	0.041	0.040	1.00
Smokey Wood	nd	nd	nd	nd
Sour Aromatics	nd	nd	nd	nd

Sour Milk/Sour Dairy	0.02	0.00	0.015	0.32
Warmed Over	nd	nd	nd	nd
Soapy	0.05	0.02	0.033	0.57
Floral	nd	nd	nd	nd
Petroleum-Like	nd	nd	nd	nd

[1]USDA Choice, marbling score Small[00] to Small[99] (400 to 499), Modest[00] to Modest[99] (500 to 599), Moderate[00] to Moderate[99] (600 to 699),
[2]USDA Select, marbling score: Slight[00] to Slight[99] (300 to 399)
[3]SEM: Standard error of the mean (largest) of the least squares means
[4]*P*-values < 0.05 are considered significant
[5]Palatability traits were reported on a 16-point scale (0 – no detection to 16 – very intense/strong)
[ab]LSMeans within a row that lack a common superscript differ ($P < 0.05$)

APPENDIX B

FIGURES

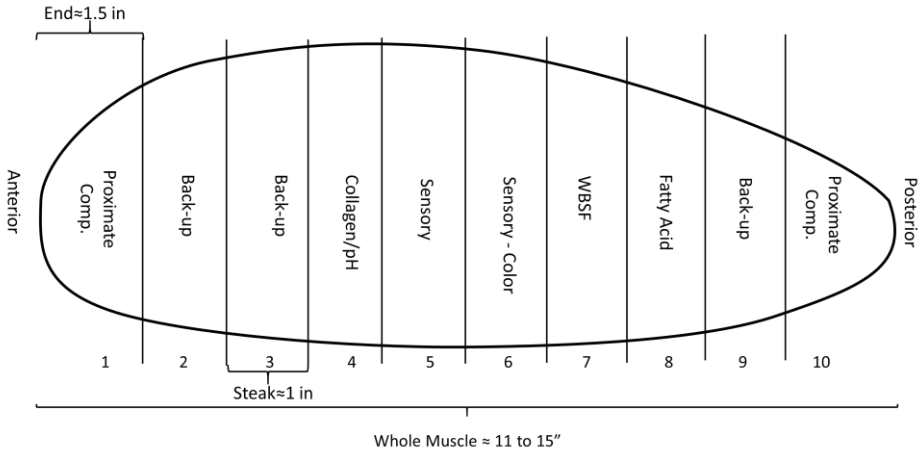

Figure 1, *M. Rhomboideus* serial cuts

APPENDIX C

POWDERING SAMPLES FOR ANALYSIS

1. Previously frozen samples were thawed until meat could be cut
2. Meat samples were hand cut into small cubes ½ inch or smaller
3. Sample was placed into a wire straining basket and lowered into a container of liquid nitrogen
4. Samples were submerged for 30 sec or until liquid nitrogen stopped bubbling
5. Frozen sample pieces were transferred to a stainless steel Waring blender and blended until a homogenous powder was formed
6. Powdered samples were transferred to a whirl pack back and stored frozen until analysis.

APPENDIX D

AOAC 992.15 LECO F-528 RAPID NITROGEN/PROTEIN ANALYSIS

Perform Leak Checks prior to running any samples

1. Press “Diagnostics”, then press “Leak Check”, Select either “Oxygen Leak Check” or “Helium Leak Check”. (Both leak checks should be performed).
2. If leak check is ok, continue on to analysis. If leak check does not pass, refer to instrument manual.

Running Blanks

1. Press “Analyze” then press “Start” to open the sample chamber, press “Start” again to close the chamber and begin blank.
2. Run blanks until protein reading is near zero (0.012 or -0.012), approximately 5 blanks
3. Check the S.D. of blanks by pressing “Results”, select at least 3 blanks by highlighting blanks and pressing “Select”, then press “Menu” and select “Statistics”. The S.D. should be ≤ 0.03.
4. Calculate blank by pressing “Calibrate”, select at least 3 blanks by highlighting blanks and pressing “Select”, then press “Menu” and select “Calculate Blank”, press “Exit”

Running Standards (Performed before new project, after bottles are changed, after maintenance)

1. Weigh ~.3500 grams of standard (EDTA) in tin foil cups, record weights (Need at least 5).

2. Press “Analyze”, enter the weight, press “Select” twice, then enter Sample ID.
3. Place standard in opening, then press “Start” to open the sample chamber, press “Start” again to close the chamber and begin analysis.
4. The first few standards will be conditioning standards, do not use for calibration.
5. Check the S.D. (or RSD) of standards by pressing “Results”, select at least 3 standards by highlighting blanks and pressing “Select”, then press “Menu” and select “Statistics”. The target S.D. is on the certificate of analysis with the standard.
6. Calculate calibration by pressing “Calibrate”, select at least 3 standards by highlighting standards and pressing “Select”, then press “Menu” and select “Calculate Calibration”, enter Nitrogen Standard value found on certificate of analysis with the standard, press “Select”, press “Yes”, press “ Exit”.
7. Recalculate by pressing “Menu” on calibration screen, press “Recalculate”, press “Recalculate Today”, press “Exit”.

Running Samples

1. Weigh ~.3000 grams of sample in tin foil cups, record weights (done in triplicate).
2. Press “Analyze”, enter the weight, press “Select” twice, then enter Sample ID.
3. Place sample in opening, then press “Start” to open the sample chamber, press “Start” again to close the chamber and begin analysis.
4. Record % Protein from screen.

APPENDIX E

AOAC 950.46 MOISTURE ANALYSIS

Equipment:
Gloves
Whatman Filter paper: #2 Qualitative Circles, 125 mm
Stapler with staples
#2 pencil
Desiccator with desiccant
Analytical balance/scale
Convection oven

**Gloves should be worn at ALL times
Procedure

1. Construct thimbles from Whatman #2 filter paper folded into a sleeve open at one end and stapled at the other end
2. Label thimbles with #2 pencil
3. Dry thimbles for a minimum of 12 hours at 100° C using an air dry oven. Oven should not be overfilled. Only 1 pan per shelf and not stacked on desiccant. Metal pans should not tough any of the walls of the oven, as air must be able to circulate.
4. Ensure desiccator is properly equipped with functional desiccant, sealant, and is not overfilled with thimbles/samples
5. Desiccator should be opened by sliding lid to remove thimble/sample and then immediately sealed.
6. Transfer dried thimbles to desiccator
7. Cool thimbles in desiccator for 30 minutes

8. Record dried thimble weight and 1 staple to the nearest 0.0001g. This is “initial thimble weight”. See #5 for opening/closing desiccator and place thimble immediately on the scale. **Record 1st weight**.
9. Put 2-3 grams of powdered homogenous sample into thimble and record the weight plus 1 staple to the nearest 0.0001 grams. This is “initial thimble/sample weight”. **Each sample should be performed in triplicate**.
10. Fold over open end of the thimble and seal with a staple.
11. Place thimble on clean metal pan. Samples should be laid flat and not overlapping.
12. Dry in 100° C dry oven for 16-18 hours. Oven should not be overfilled. Only 1 pan per shelf and not stacked on desiccant. Metal pans should not tough any of the walls of the oven, as air must be able to circulate.
13. Cool in desiccator for at least 1 hour. #4 should still be true.
14. Record dried thimble weight and 1 staple to the nearest 0.0001 gram. This is “dried thimble/sample weight”. See #5 for opening/closing desiccator and place thimble immediately on the scale.

APPENDIX F

AOAC 960.39 ETHER EXTRACT/FAT ANALYSIS

Equipment:
Gloves
Desiccator with desiccant
Analytical balance/scale
Convection oven
Soxhlet apparatus
Fume hood
Boiling flask
Glass boiling beads/chips
Tongs
Funnel

Reagents:
Petroleum Ether

**Gloves should be worn at ALL times

1. Ensure new petroleum ether is present, as well as properly labeled hazardous waste container for used petroleum ether
2. Turn on chilled water system. Check drain to ensure water is passing through condenser system. Water flow should be sufficient to cool condenser yet slow enough for drain.
3. New petroleum ether can be used, as well as used or distilled petroleum ether that is still clear and not yellow. If petroleum ether is yellow, either can be distilled or discarded into hazardous waste container
4. Insert no more than 15-18 dried thimbles/samples per Soxhlet. Thimbles should be inserted below the 1st loop of the Soxhlet for optimum fat extraction
5. Add glass boiling beads to each boiling flask.
6. Under fume hood, add enough petroleum ether (approx.. 1L) to just above the 100mL mark on the flask

7. Assemble flask, Soxhlet, and condenser with unit being in a vertical position.
8. Cover joints with parafilm.
9. Set temperature control on heat mantles to 3.5 to 4 or to appropriate setting for a petroleum ether drip rate of 4-6 drops/second
10. Turn on mantles, and mark beginning time in daily ether extraction log.
11. Stay in lab to ensure: ether begins to boil, condensers are cool; ether rate is within 4-6 drops/second; assure either unit is filling and recycling correctly; parafilm is secure and ether is not leaking; hoods are working at 90-110% flow rate with 100% flow rate optimum.
12. **Ether extraction unit must be checked every hour and a designated person MUST be in building during their watch. A person MUST always be present within the building checking ether extraction unit on a hourly basis. IT IS UNACCETABLE TO LEAVE THE BUIDLING WITH ETHER EXTRACTION UNIT UNATTENDED.**
13. When checking the extraction unit every hour, the following must be done:
    a. Chilled water is still running through condensers properly
    b. Soxhlets are still at the 4-6 drops/second rate and recycling
    c. Parafilm is intact and not compromised
    d. Ether is not being evaporated
    e. Hood is still operational at 90-110% flowrate with 100% flowrate optimum
    f. Fill out daily ether extraction log

14. Samples should be extracted for 18 **continuous** hours
15. After extraction period, mantles should be turned off and action recorded in daily ether extraction log
16. Allow Soxhlet apparatus to cool (approx. 30 minutes and ether is no longer boiling)
17. Disassemble the Soxhlet apparatus. Condensers should not be removed from clamps, but slid up and down.
18. Place flask in cork ring under hood and pour excess ether from Soxhlet into flask.
19. Place samples on clean metal pan lying flat and not overlapping each other in hood until ether is evaporated (approx.. 1 hour or until no ether odor is present). Hood flowrate should be between 90-110% with 100% flowrate being optimum. If hood is not at desired flowrate samples should be tranfered to an operational hood.
20. After 30 minutes since mantle turn off, shut off chilled water.
21. Transfer "extracted thimble/sample" in desiccator to 100°C air dry oven. No ether should be present in thimbles before samples are placed in oven. **FAILURE TO DO SO WILL RESULT IN AN ETHER FIRE. Oven should not be overfilled. Only 1 pan per shelf and not stacked on desiccant. Metal pans should not touch any of the walls of the oven, as air must be able to circulate.**
22. "Extracted thimble/sample" should be dried for 12 hours

23. After 12 hours, remove samples and place into desiccators for 1 hour or until cool. Desiccator should be opened by sliding lid to remove thimble/sample and then immediately sealed. Ensure desiccator is properly equipped with functional desiccant, sealant, and is not overfilled with thimbles/samples
24. Record weight of “extracted thimble/sample” to the nearest 0.0001gram.

APPENDIX G

MODIFIED FOLCH METHOD FOR FATTY ACID ANALYSIS

Equipment:
Per sample: (2) 50 ml Test tubes, (2) 20 ml test tubes, (1) scintillation vial, (1) gas chorography vial
Whatman filter apparatus & Filter Paper
Reagents:
Chloroform: Methanol 2:1 (Chloroform is a known carcinogen, use caution. Avoid inhalation of fumes and always wear gloves and face respirator
0.74% KCL
0.05% N KOH in MeOH
14% BF3 in MeOH
NaCl
Hexane

Extraction of Total Lipids

1. Weigh 500 mg adipose tissue or 1-5g muscle and add to labeled 50-mL glass tube. (more can be used but only saponify 100µL of the extracted lipid.)
2. Add 5mL of chloroform:methanol (CHCl3:CH3OH. 2:1, v/v)
3. Homogenize each sample with Polytron homogenizer on medium setting for 30s. After homogenization, rinse the probe with CHCl3:CH3OH until you have a final volume in the tube of 15mL.
4. Rinse probe in warm water. Spray with clean water into waste beaker. Rinse probe with CHCl3:CH3OH into another waste beaker. Dry with Kimwipes.
5. Let sample sit for 30min to extract lipids. If stopping at this point, flush with nitrogen, cap and store in cooler.
6. Filter homogenate through sintered glass filter funnel (or Whatman filter apparatus using 2.4cm GF/C filters) into a 2nd 50mL centrifuge tube

7. Rinse 1st tube 2-3 times with CHCl3:CH3OH. Also, rinse the filter funnel 1-2 time with CH3Cl:CH3OH.
8. Q.S. filtered solution homogenate to convenient volume (20-30mL)
9. Add 8mL of 74% KCl and vortex 1 min
10. Let sit 2hrs to separate phases or centrifuge (20-30min) until you get two distinct phases. If stopping at this point then flush with nitrogen, cap , and let sit in the refrigerator overnight.
11. Carefully remove the lower phase, transfer all lower phase to scintillation vial. If you want to stop at this point, then flush with nitrogen, cap and store in -20° C.
12. Evaporate the sample to dryness (weight SV/lipid) with nitrogen using the N-Evap at 40° C.

    *Transfer dry lipid from SV to 20mL test tube w/ 5mL of Chloroform:Methanol then wash out 2 times with 5 mL → total 15mL

Saponification and methylation of Lipids

(If you used 0.5g adipose tissue or other lipid source, only saponify 100µL of lipid)

1. Add 1 mL of 0.5 N KOH in MEOH; heat in 70° C water bath for 10 min. This separates FA's
2. Add 1 mL of 14% BF3 in MeOH; Flush with N2; loosely cap; place in 70oF water bath for 30 min. Allow time to cool (10 mins). This attaches methyl to FA
3. This procedure saponify the lipids, i.e., it liberates the fatty acids from the glycerol backbone. The fatty acid is methylated in the process, removing the net negative charge of this group.

4. Remove the tubes and allow them to cool. Add 2 mL HPLC grade hexane and 2 mL saturated NaCl; vortex 1 min; centrifuge 10 min.
5. Pipet off upper layer with transfer pipette; place in 20 mL glass scintillation vial containing 800 mg NaSO4. Add 2 mL of hexane to the tube containing saturated NaCl; vortex, allow phases to separate, and pipette the upper hexane layer into the 20 mL tube with NaSO4. You should have now 4 mL of hexane in this tube. Vortex briefly. The NaSO4 removes any moisture in the hexane.
6. Pipet the hexane into a labeled 20 mL test tube
7. Add 1 mL hexane to the 20 mL vial with NaSO4 in it. Vortex briefly. Transfer this hexane to the test tube.
8. Evaporate completely with N-Evap set at 40° C.
9. Reconstitute the lipid with the appropriate amount of hexane to obtain approximately 50mg/mL.
10. Bring up samples as follows:
11. 500µL hexane for plasma and digesta
12. 1mL hexane for adipose tissue
13. Pipet 400µL of this solution into a 2 mL autosampler vial containing 1.6 mL of HPLC grade hexane. Only dilute adipose tissue samples. For plasma and digesta use a glass insert and pipet 100µL undiluted to run on the GC

Solution Preparation:

0.5N KOH in MeOH
2.81g KOH
100mL of MeOH
Saturated NaCl31.7g NaCl
100mL ddH2O
0.74% KCL
7.4KCL
1L ddH2O

APPENDIX H

COLLAGEN CONTENT ANALYSIS

Equipment

50 mL disposable centrifuge test tubes
1 L volumetric flask
Glass rod(s)
Cooking jars w/ lids and seals
Centrifuge
Convection Oven
Volumetric flasks 500 mL/ 200 mL
#2 filter paper
50 mL test tube
Water bath
96-well microplate
UV-Visible Spectrophotometer
Perchloric acid fume hood

Reagents

12N hydrochloric acid
0.025 g hydroxyproline powder
250 mL .001 N HCl
7.0gNaCl
0.026g $CaCl_2$
0.35g KCl
30.0 g citric acid monohydrate
15.0 g NaOH
90 g sodium acetate trihydrate
dissolved in 500 mL ddH_2O
355 mL 70% isopropanol
1.41 g chloramine T
100 ml of buffer solution
10g 4-dimethylbenzaldehyde
35mL of 60% perchloric acid

1 liter of ddH_2O

Separation of Raw Soluble and Insoluble Collagen

1. Sample frozen in liquid nitrogen and powdered in blender

2. Duplicate samples (3.0-4.0g), record exact weight to 0.0001 gram

3. Samples placed in 50 mL disposable centrifuge test tube, add 12mL of ¼ strength Ringer's solution. Stir each tube 10 times with glass rod, cap, and cool 4° C for 15 min.

   *Ensure clean glass rod is used for each sample.
4. Centrifuged for 20 min at at 21,525 x g at 2° C
5. Decant supernatant into labeled cooking jar
6. Add 8 mL of ¼ strength Ringer's solution to residual and stir 10 times
7. Repeat #4 and #5, decanting supernatant into same cooking jar
8. Add 5 mL of ddH_2O to residual meat pellet and mix well. Pour mixture into separate labeled residual cooking jar
9. Rinse test tube with 4mL ddH_2O and add to residual jar, Wipe tube clean and any residue was added to the residual pellet jar.
10. 25 mL of 12N HCl acid is added to each supernatant
11. 25 mL of 6N HCl acid is added to each residual
12. Seal jar(s) and cook in oven under hood at 105° C for 16h
13. Remove lids and cool under hood to allow vapors to escape (approx. 1 hour).
14. Transfer to volumetric flasks (200 mL for supernatant hydrolysates and 500mL for residual), bring to volume of 200 mL and 500 mL with ddH_2O respectively).
15. Filter through #2 filter paper in 50 mL test tubes. Discard remaining liquid

Standard Curve Stock Solution

16. Dissolve 0.025 g of hydroxyproline powder into 250 mL of 0.001 N HCl.

Five standard solutions: 2 mL hydroxyproline stock and 98 mL ddH_2O, 4 mL hydroxyproline stock and 96 mL ddH_2O, 6 mL hydroxyproline stock and 94 mL ddH_2O, 8 mL hydroxyproline stock and 92 mL ddH_2O, 10 mL hydroxyproline stock and 90 mL ddH_2O=

17. Add 2 mL of each standard solution into duplicate test tubes, along with two blank tubes of 2 mL ddH_2O. 2 mL of each of the filtrates are added to individual test tubes.
18. Add 1 mL of an oxidant solution

    *pH should be adjusted to 6.0 with NaOH
19. Vortex and rest for 20 minutes at room temp
20. 1 mL of color reagent is added
21. Vortex and cover tubes with aluminum foil and place in 60° C water bath for 15 min. Uncover and cool to room temp
22. Transfer to 250 microliter to 96-well microplate. Read with Epoch microplate spectrophotometer at 558 nm absorbance. Read blanks and standards first

23.

Solution Preparation:

Diluted 6N HCl

1:1 ratio of 12N HCl: ddH_2O

¼ Strength 250 mL Ringer's stock

7.0gNaCl
0.026g $CaCl_2$
0.35g KCl

Buffer Solution

30.0 g citric acid monohydrate
15.0 g NaOH
90 g sodium acetate trihydrate
dissolved in 500 mL ddH_2O
290 mL isopropanol

Oxidant Solution

1.41 g chloramine T
100 ml of buffer solution

Color Reagent (Ehrlich's solution)***

10g 4-dimethylbenzaldehyde
35mL of 60% perchloric acid
65 mL of isopropanol is added slowly to the mixture
*****Performed under perchloric acid approved fume hood**

APPENDIX I

HUNTER LAB MINI SCAN XE PLUS STANDARD OPERATION PROCEDURES

** Always handle the black and white standardization plates with care. Do not scratch or chip them.

1. Plug Mini Scan into electrical outlet.
2. Wrap PVC overwrap over aperture insuring a smooth, tight fit. Also wrap the black and white standardization plates with PVC overwrap. Make sure there are no air bubbles or wrinkles on the surface of the plates where the readings will be taken.
3. Wipe the black plate with a Kimwipe to insure it is clean and place the black plate on the circle of the calibration tile holder.

4.Place the Mini Scan on the calibration tile holder so the two rubber feet are in the two holes of the holder and the aperture is centered on the black plate. The aperture should fit flatly on the black plate to insure that there is no interference when taking readings.

5. Push the lightning bolt key on the Mini Scan to turn the unit on.
6. Make sure that the XYZ values on the screen correspond to the XYZ values listed on the back of the white plate.
7. You are now ready to standardize the unit. Press the lightning bolt key and the Mini Scan will read the black plate.
8. When the reading is complete, the screen will indicate that the machine is ready to read the white plate.

9.Remove the black plate from the calibration tile holder and replace it with the white plate. Wipe the white plate with a Kimwipe. Make sure that the aperture of the Mini Scan sits flatly on the white plate.

10. Press the lightning bolt key to read the white plate.
11. Press the lightning bolt key three times and the MiniScan will be ready to read the first sample.

Procedure to Record L* a* b* Color Scores

1. Use left and right arrow keys to select the appropriate setup.
2. (For my research Daylight Color was used with a 10 degree observer)
3. Position the aperture of the Mini Scan on the part of the meat sample to be tested. Be sure that the aperture fits flatly on the meat but do not apply pressure. The spot to be tested should be representative of the steak muscle tissue. There should not be a lot of connective tissue, seam fat or subcutaneous fat where the color reading is taken.
4. To take a reading, press the lightning bolt key.
5. Record the L*a*b* values and press the lightning bolt key again to take a second reading of exactly the same spot on the meat sample.
6. Record the L*a*b* values and take a third reading by pressing the lightning bolt key.
7. Record the L*a*b* values.
8. The Mini Scan is now ready to read the next sample. Repeat the process. Before taking readings on the second meat sample, make sure that the PVC overwrap covering the aperture is clean and free of fat or anything that might interfere with a clean reading.

9. Be sure that the Mini Scan is clean and that the aperture is clean before putting the machine away.

APPENDIX J

MEAT PH MEASUREMENT PROCEDURE

Equipment:
Blender
Pint Jars
pH meter with pH electrode
Stir plate
Magnetic stir bars
Reagents:
Distilled water
Buffer, pH 4.0 and pH 7.0

Procedure:

1. Place approximately 10 g of the frozen powdered sample into a pint jar.
2. Add 90 g distilled water to the pint jar, attach blender blade, o-ring, and screw cap. Blend on high speed for 15 to 20 seconds to make a smooth slurry.
3. Place a magnetic stir bar in the bottom of the jar and place on stir plate. Stir plate should be moderately agitating the sample (~200 RPM) when the probe is lowered into the sample jar.
4. Measure the pH of this slurry with a pH meter that has been calibrated with two standard buffer solutions. One buffer at pH = 7.0 and the other (either 4 or 10) having a pH value near that of the final.
5. The electrode should be placed in the stirred slurry for about 30 seconds to allow the electrode to equilibrate.
6. Press read to begin pH measurement. “Stable” will appear when reading is finished. Record the pH of the slurry after the electrode has stabilized.

7. Do NOT leave the pH probe in the meat slurry. Remove the pH probe from the slurry and wash it thoroughly with distilled water. Be sure to gently wipe all fat and connective tissue from the probe.
8. Always store the pH probe in CLEAN distilled water or pH 7 buffer.
9. NEVER let the bulb dry out

APPENDIX K

COOKING AND WARNER-BRATZLER SHEAR FORCE

Equipment
Scale
Flat top countertop electric griddle
Thermocouples
Threading needle
Thermometer
Surface infrared thermometer
Plastic soufflé cup
1.3-cm diameter corer
500-kg load cell
1.02-cm V-shape blade (60^{o} angle)

Cooking Procedures

1. Record initial raw weight to the nearest 0.01 gram.
2. Insert threading needle into geometric center of raw tempered steaks
3. Thread thermocouple through needle, and remove needle
4. Insert needle <0.635-cm from the first insertion point and repeat #2.
5. Pull thermocouple (second insertion), until end is approximately in center of steak and pull excess slack from thermocouple.
6. Record initial temperature in Celsius
7. Place steaks on gridle (pre-warmed to 180°C) and record initial temperature
8. Monitor steak internal temperature. Flip steak upon reaching internal temp. 35° C.
9. Check surface temperature often with surface infrared thermometer to ensure consistent 180° C cooking surface
10. Remove steaks from griddle upon reaching internal temperature 71° C and record time/temperature.

11. Remove thermocouple and place cooked steak on scale and record cooked weight.

Sensory Panel Testing

1. Panelist are served two “warm-up” sample to insure proper scaling and precision of scoring. Using a knife, external edges of steaks will be removed and 1.3-cm x 1.3 cm x steak thickness cubes will be cut.
2. 2 cubes are placed in plastic cups and served to panelist with 4 minutes between each sample.

   *A break of 15 minutes will be taken after the sixth sample

Warner-Bratzler Shear Force

1. Post-cooking steaks will be allowed to chill under refrigerated conditions (<4° C) for a minimum of 24 hours.
2. Using a knife, external edges of steaks will be removed and a 1.3-cm core will be taken
3. Cores should run parallel to the muscle fibers, and avoid excess deposits of fat
4. Cores will be placed beneath V-shaped blade
5. Blade crosshead speed is set at 200-mm/min using a 500-kg load cell.
6. The peak force (kg) is recorded.