Prediction of Toughness and other Beef Quality Characteristics of the m. *Longissimus Thoracis* using Polarized Near-Infrared Reflectance Spectroscopy

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Food Science and Technology

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Abstract

Near-infrared (NIR) spectroscopy using polarized light was used to predict Warner Bratzler Shear Force (WBSF), Commission de l'Eclairage (CIE) colour variables L*, a*, b*, drip loss, cooking loss, pH and intramuscular fat (IMF) of bovine longissimus thoracis (LT) muscle. Muscles were derived from a 2 × 2 × 2 factorial study investigating the effects of residual feed intake (RFI) (low versus high, n = 27 and 21, low and high RFI, respectively), hormonal growth promotants (control versus implanted, Component E-S followed by Component TE-S, n = 24), β-adrenergic agonist use (control versus ractopamine hydrochloride, 200 mg/head/day, last 28 days of finishing, n = 24) on the LT meat quality of crossbred Angus steers at 3 and 12 days post mortem. Analysis of variance indicated that LT from HGP steers had a greater mean WBSF value at day 3 post-mortem (57.04 ± 1.82 kg) than non-implanted steers (45.14 ± 1.78 kg), with the difference in WBSF between the treatments declining with aging as there was no significant difference in mean WBSF value due to HGP by day 12 post-mortem. Supplementation with RAC did not affect WBSF values at the level used (200 mg/steer/day) (p=0.74). Post-mortem aging reduced cooking time and increased L*, a* and b* values ($p < 0.01$). Mean L* value for low RFI steers was significantly greater than that of high RFI steers ($p = 0.007$). The RFI x HGP x RAC interaction was significant for intramuscular protein ($p = 0.002$), with HGP treatment increasing protein content of high RFI steers fed with RAC.

Near-infrared spectra were collected at days 3 and 12 post-mortem without (N) and with a polarizer (1100 to 1650 nm) with its axis perpendicular (R) and parallel to the muscle fiber direction (P). Partial least square regression indicated that spectra collected in R mode at day 3 post-mortem gave the best validation r2 for WBSF (0.81 ± 0.01), drip loss (0.74 ± 0.02) and

cooking loss (0.85 ± 0.008). Colour measurements L* and a* were best predicted in P mode (r2 = 0.76 ± 0.02; 0.83 ± 0.01) while b* was predicted with more than 80% accuracy with or without polarization at day 3 post-mortem. IMF (r2 = 0.81 ± 0.01) and pH (0.79 ± 0.03) were best predicted with a polarizer, with no difference in P and R mode at day 3 post-mortem. At day 12 post-mortem, colour variable L* was best predicted in P mode (0.82 ± 0.006), a* was best predicted in R mode (0.72 ± 0.03) and b* was best predicted in N and P mode (0.80 ± 0.01); WBSF (0.76 ± 0.01), drip loss (0.73 ± 0.01), cooking loss (0.73 ± 0.02), and pH (0.84 ± 0.02) were best predicted in configuration R and IMF was best predicted in P mode (0.78 ± 0.01). The results obtained indicated the possibility of an efficient non-invasive method to predict meat toughness and other meat quality characteristics using polarized NIR spectroscopy.

Preface

This thesis is an original work by Devesh Sharma. The research project, of which this thesis is a part, uses animals that were managed according to the University of Alberta's animal care and use program which meets the national standards sets by Canadian Council of Animal Care (CCAC). The study was reviewed by a University of Alberta animal care and use committee prior to its execution (Animal Use Protocol number AUP00001801).

Acknowledgment

I am thankful to Dr. Heather Bruce for her valuable supervision to complete this thesis. Her guidance on research, courses and my career throughout the span of my programme has been priceless. Her positive attitude, and clear conscience has been a consistent source of support and motivation for me. I appreciate the great opportunity that she provided me to be her student and the knowledge and concepts I've learned from her in the area of meat science and statistical analyses without which this research would not be possible.

I am grateful to Dr. Abdulhakem Elezzabi for being a member of my supervisory committee and for his guidance, constructive criticism in the area of light spectroscopy, and his valuable inputs in my thesis.

I am thankful to Dr. Faranak Teimoory who helped in collecting data for this research and who provided me with tools that were useful in completing this thesis.

I acknowledge the moral support of my family, all my colleagues, and friends.

Finally, I would like to thank Alberta Livestock and Meat Agency Ltd. (ALMA), Alberta Innovates Bio Solutions, and the NSERC CREATE Meat Education and Training Network (MEaTnet) for their financial support of this project and my involvement in it.

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Abbreviations

ADG	Average daily gain
AOAC	Association of Agricultural Chemists
ATP	Adenosine triphosphate
β-AA	β-adrenergic agonists
BW	Body weight
CCP	Critical control points
CCAC	Canadian council of animal care
CP	Crude protein
CPK	Creatine phospho-kinase
COMBO	steers treated with both hormonal growth promotants and β-Adrenergic agonist
CONT	control treatment
CSA	Cross-sectional area
DFD	Dark, firm and dry
DMI	Dry matter intake
DOA	Day of aging treatment
FDA	Food and drug administration
FFA	Free fatty acid
FT	Fourier transformation
G:F	Growth to feed ratio
GP	Growth promotants
HCW	Hot carcass weight
HGP	Hormonal growth promotants

IMF	Intramuscular fat
IMCT	Intramuscular connective tissue
LL	*Longissimus lumborum*
LT	*Longissimus thoracis*
MLR	Multiple linear regression
MSA	Meat Standards Australia
MSC	Multiplicative scatter correction
NIR	Near infrared spectroscopy
N	Spectra collected without a polarizer
OSC	Orthogonal signal correction
P	Spectra collected with polarizer when polarizer axis was parallel to the muscle fiber direction
PCA	Principal component analysis
pH_{1h}	pH measured at hour one post-mortem
pH_{3h}	pH measured at hour three post-mortem
PLS	Partial least square
PLSR	Partial least square regression
R	Spectra collected with polarizer when polarizer axis was perpendicular to the muscle fiber direction
RAC	Ractopamine hydrochloride
RFI	Residual feed intake
r^2	Coefficient of determination of prediction
R^2	Coefficient of determination of calibration
SNV	Standard normal variate

SG	Savizkye-Golay
SSF	Slice shear force
T_{1h}	Temperature measured at hour one post-mortem
T_{3h}	Temperature measured at hour three post-mortem
TBA	Trenbolone acetate
TPA	Texture profile analysis
UTM	Universal testing machine
VIS	Visible
WBSF	Warner Bratzler shear force
WHC	Water holding capacity
ZH	Zilpaterol hydrochloride

Chapter 1: Literature Review

Introduction:

Prediction of consumer acceptance of meat has been the subject of extensive research (Hildrum et al., 1994; Hildrum et al., 1999), as the acceptability of a product by consumers is the gold standard by which product marketability is measured. The use of consumer or even trained panels can be costly and time consuming, however, and this prompted the development of objective measures to provide some level of indication of consumer acceptability. Objective measures were developed for important meat quality characteristics such as the Warner-Bratzler shear force (WBSF) test for meat toughness, adoption of the Minolta colour meter for description of colour, and drip loss and water holding capacity assessments (Honikel, 1980) to predict product juiciness. In recent decades, there has been an impetus to develop non-invasive, non-destructive technologies to measure and predict the eating quality of meat (Vote et al., 2003; Andres et al., 2008; Wu et al., 2012). The use of non-invasive, non-destructive technologies would reduce product and also revenue currently lost to meat quality assessment by consumer panels, WBSF, drip loss analysis and other quality measurements. Current technologies used in the beef industry predict composition only, and include digital imaging, which measures visible fat contents, and near-infrared spectroscopy (NIR), which is used commercially to predict moisture, fat, protein and collagen content of beef. Recently, digital imaging algorithms have predicted the toughness of cooked beef with over 90% accuracy (Wu et al., 2012), but NIR has shown significant promise as a non-invasive, non-destructive technology that requires fewer computing resources than digital imaging. Refining NIR to become an effective predictive technology for beef quality requires an understanding of what defines the eating quality of meat, and what factors affect and determine beef quality. With this literature review, the research

performed in the past will be reviewed, and the research needed to develop NIR spectroscopy as a technology for estimating the eating quality of beef will be identified.

Consumer perceptions of meat quality:

Consumer quality evaluation models distinguish between the product as a food (safety, nutrition, sensory, and ethical) and the product as a trade object (certification, traceability, convenience, and price) (Peri, 2006). As a food, consumer quality evaluation of meat may be divided into two phases, with the first phase occurring before the purchase of the product and the second phase occurring after the purchase of the product (Grunert et al., 1996). In the first phase, quality assessment is based on the intrinsic and extrinsic characteristics of the meat. Intrinsic quality cues are those which are physically part of the product itself and include muscle colour, fat colour, and degree of marbling, while extrinsic cues are not physically part of the product and include country of origin, price, place of purchase, presentation of a product and brand value, and packaging) (Troy and Kerry, 2010).

Appearance is the main factor used by consumers during quality assessment and the decision to purchase red meat (Kristina, 2000). Assessment of meat quality based on appearance includes consideration of factors such as colour, purge loss in the tray, drip loss on the surface of the meat, the degree of marbling, and the texture of the raw meat perceived by viewing the meat surface iridescence (Kropf et al., 1992; Becker, 2000). Colour is often used as a cue of the freshness of meat by customers (Adams and Huffman, 1972). Meat colour is affected by the concentration and level of oxygenation of myoglobin in the meat, myoglobin being the main pigment containing protein in meat, as the oxidative state of the heme ring within myoglobin and

compounds attached to myoglobin determine the reflectance wavelengths associated with the meat. As the level of myoglobin increases with age, meat from mature animals appears darker red than meat from young animals (Miller, 2002)

Although meat colour is the first factor perceived by the consumer that influences their purchasing decision, it may not represent the overall eating quality of meat. In a study by Carpenter et al. (2001), colour influenced the purchasing decision of customers with red meat being more desirable to consumers than brown, but the colour did not bias eating satisfaction. Wulf et al. (2002) reported no difference in the juiciness and flavor intensity of steaks obtained from dark and normal beef. In a study of Viljoen et al. (2002), consumers preferred bright red raw steaks more than dark red raw steaks but reported no difference in flavor, texture, and juiciness between the dark and normal steaks when the steaks were cooked. Also, dark beef with a low pH does not have a flavor different from that of normal beef (Viljoen et al., 2002; Holdstock et al., 2014), although dark beef with a high pH has been shown to have a different flavor than normal beef (Holdstock et al., 2014).

After purchase, Kristina (2000) reported “tenderness” and “flavor” to be the most important quality characteristics of beef in almost every European country while “leanness” and “free of gristle” were of least importance. Grunert (1997) found taste, juiciness, freshness and leanness as the most important quality characteristics of beef by consumers in France, Germany, Spain and the United Kingdom. The relative importance of quality characteristics may change with sex and age of the consumer, with men responding more to texture than women, and there being an increased contribution of flavor in the perception of overall quality with consumer age

(Schutz and Wahl, 1981). In a study conducted by Schutz and Wahl (1981), 420 respondents scored flavor as the greatest contributor to the overall quality of meat, greater than appearance and texture, with the latter two attributes similar in their relative importance to the overall quality of the meat. Extrinsic cues may play a role but are not significant, with the price being the least determinant quality assessment factor by respondents in all the European countries being studied, specifically the U.K., Italy, Ireland, Germany, Sweden and Spain (Kristina, 2000).

Apart from intrinsic and extrinsic quality characteristics, meat safety concerns may negatively affect the purchasing decision of consumers. Kristina (2000) reported European consumers are more concerned about the use of growth promotants and antibiotics, salmonella occurrence and BSE than fat/cholesterol. However, external fat on beef table cuts and seam fat of chuck and round reduces the value of beef in the perception of American consumers (Unnevehr & Bard, 1993). Issanchou (1996) also reported negative consumer perception for visible fat.

Factors affecting meat quality

The intrinsic characteristics of muscle affecting consumer perception of beef quality are affected by many factors, some of which may be manipulated by controlling animal characteristics through genetics or the management of the animal pre-slaughter, and through early post mortem carcass processes. For example, age at slaughter is related to the eating quality of meat, with a negative correlation between animal age and cooked beef tenderness (Martin et al., 1971), presumably due to the influence of the heat stability of collagen (Hill, 1966), which increases with animal age due to increased heat stability of the collagen crosslinks (Roy et al. 2015).

Meat quality differences may also be attributed to muscle fibre characteristics and ultimate pH of the meat, which can directly affect meat tenderness and muscle colour. Muscle fibre type can affect meat quality through its morphology such as the number of fibres within a muscle, the cross-sectional area of the muscle fibres and by the proportion of the various muscle fibre types (type I, type IIA, type IIX, type IIB) as their combination in a muscle determine its contractile and metabolic properties (Ryu et al., 2005; Lee et al., 2010; Joo et al., 2013). Muscle fibre composition also affects the post-mortem metabolic rate, with an increase in the percentage of type IIb fibers related to increased post mortem metabolic rate, which can be a major determinant of meat quality (Ryu et al., 2005) and muscle colour (Bruce and Ball, 1990). Increased proportion of type I muscle fibres has been associated with decreased colour stability and the formation of metmyoglobin, which leads to an undesirable browning of meat (Renerre, 1990). Type IIB fibres have been related to light red meat with lower water holding characteristics (Kim et al., 2013). Tenderness however has been positively correlated with increased proportions of type I muscle fibres and negatively correlated with increased proportions of type II fibres (Hwang et al., 2010). This may occur because an increased proportion of type I fibres has been reported to be associated with a decrease in the rate of anaerobic glycolysis early post-mortem and thus a decrease in the rate of early post-mortem intramuscular pH decline (Choi et al., 2007). A coarse appearance of the muscle is related to the size of the muscle fibres, which affects the size of the muscle fibre bundles (Kim et al., 2013; Rehfeldt & Kuhn, 2006; Ryu & Kim, 2005), and muscles with large fibre size (especially fibre type IIB) have been more often associated with tough meat than muscles with small fibre sizes (Renand et al., 2001). Type I fibres are also positively correlated with IMF, especially increased phospholipid concentration, which positively affects cooked meat flavor and juiciness (Maltin et al., 1998; Hwang et al., 2010).

The total number of fibres and cross-sectional area of fibres are controlled by sex hormones, as they are androgenic, particularly in cattle. Testosterone has been reported to stimulate muscle hypertrophy without changing the total number of fibres through satellite cell proliferation and muscle protein synthesis (Yoshioka et al., 2007). The profound androgenic effect of sex hormones in cattle prompted their use as growth promotants.

Hormonal growth implants have been used in beef production for more than 45 years (Zobell et al., 2000). Hormonal growth promotants (HGP) are extensively used in beef production to raise cattle quickly, to gain additional weight with the same or less feed (increased average daily gain), to increase carcass weight, and to reduce fat deposition (Smith et al., 2007; Duckett and Pratt, 2014; Chikhou et al., 1993; Avendaño-Reyes et al., 2006; Winterholler et al., 2008). HGP are pharmaceutical drugs that work as a normal growth hormone in an animal, and they are implanted between the skin and cartilage behind the ear from where they can slowly release the drug into the blood of an animal. They are extensively used in the beef industry as they not only improve feed efficiency, they also decrease production cost and produce leaner meat (Duckett and Pratt, 2014). In the United States, 90% of feedlot-finished slaughter cattle have received at least one implant in their lifetime (Johnson, 2015), and HGP can improve the feed efficiency of beef cattle by 15-25% (Hutcheson, 2008). Implants containing trenbolone acetate (TBA) and estradiol may yield a profit of $163 per head to cattle producers (Duckett and Pratt, 2014). In Canada, two types of growth implant are approved for use: those containing natural hormones (progesterone, estradiol benzoate, testosterone propionate) such as Synovex S and Synovex H, which are reported to increase the feed efficiency by 10-15%; and those containing chemicals

with hormone-like activity such as zeranol, which has estrogenic activity, trenbolone acetate, which has androgenic activity, and melengesterol acetate, which has progesterone-like activity. .Apart from decreasing the production cost, use of growth promotants may affect meat quality. HGP implants were originally not considered in the critical control points (CCP) developed by Meat Standards Australia (MSA), which aimed to identify all the CCPs from the various stages of cattle production and processing that can influence beef palatability. Subsequent research indicating the adverse effects of implants on beef quality (Roeber et al., 2000) prompted the later addition of HGP implants as a CCP in the model to predict beef palatability (Thompson, 2002).

Another category of growth promotants is that of the β-adrenergic agonists (β-AA). Relative to steroid growth promotants , β-adrenergic agonists (β-AA) are a comparatively new growth promotant and are administered as a feed supplement. β-AA are synthetic compounds which, once absorbed in the blood, bind to β-adrenergic receptors on the cell surface and stimulate those receptors to promote muscle growth in an animal (Mersmannn et al., 1998). β-AA are added to feed during the last 28 to 42 days of the finishing period of cattle. Type II muscle fibres are more sensitive to β-AA, and this leads to increased muscle fibre cross-sectional area in these muscle fibres which, in turn, is responsible for the increase in muscle mass observed (Miller et al., 1988; Smith et al., 1995; Johnson et al., 2014). β-AA operates as repartitioning agents primarily through increasing muscle protein deposition (Leheska et al., 2009), which increases muscle hypertrophy. Muscle hypertrophy also is facilitated by upregulation of the protein calpastatin, which binds to and inhibits the proteolytic calpain enzymes, thereby decreasing muscle protein hydrolysis (Wheeler and Koohmaraie, 1992). β-AA also reportedly decrease the proportion of trimmed fat and increase lean (Moloney et al., 1990; Kellermeier et al., 2009). Popular and well-

documented types of β-AA include ractopamine hydrochloride (RAC), zilpaterol hydrochloride (ZH), clenbuterol, L-644,969, and cimaterol, all of which were reported to increase carcass weight gain and feed efficiency in beef cattle (Avendaño-Reyes et al., 2006; Winterholler et al., 2007; Lean et al., 2014; Johnson et al, 2014). Of these, in Canada and the United States, RAC and ZH are the only two β-AA that are labeled for use as a feed additive for cattle by the United States Food and Drug Administration (FDA, 2003; FDA, 2006) and the Veterinary Drug Directorate of Canada. Avendaño-Reyes et al. (2006) reported a mean increase in hot carcass weight (HCW) of 22 kg when beef steers were treated with ZH and of 14 kg when beef steers were treated with RAC, when compared with control steers. Increases in average daily gain (ADG), gain to feed (G:F) ratio and HCW in β-AA treated steers is dependent upon the dose and duration of the β-AA supplemented (Pyatt et al., 2013). Bittner et al. (2016) reported mean G:F ratio increased from 5% to 13% when the RAC dose was increased from 100 to 200 mg/day/steer. The duration of β-AA treatment is an important consideration as prolonged exposure of β-AA during the end of the finishing period may lead to downregulation of β-AA receptors and in turn diminish cattle performance response (Moloney et al.,1991; Chikhou et al., 1993a). Bittner et al. (2016) also reported no effect on final body weight (BW), ADG, dry matter intake (DMI), or G:F ratio when the duration of RAC treatment was increased from 28 days to 35 days and 42 days. Increase in the duration of RAC supplementation may even lead to decreased carcass performance (Greenquist et al., 2007). On the contrary, Abney et al. (2007) reported increased HCW with an increase in the duration of RAC supplementation, with 5.7 and 2.9 kg heavier carcasses when the treatment increased from 28 days to 35 days and from 28 days to 42 days, respectively.

Both HGP and β-AA have been related to increased calpastatin levels, which can lead to reduced calpain activity and, in turn, may increase beef toughness (Roeber et al., 2000; Platter et al., 2003; Avendano-Reyes et al., 2006; Gruber et al., 2008; Strydom et al., 2009). Ebarb et al. (2016) suggested growth promotants may increase WBSF by increasing muscle fibre cross-sectional area as WBSF was positively correlated with cross-sectional area (CSA) of all fibre types at day 3 post-mortem (Ebarb et al., 2016), which agreed with the results of Crouse et al. (1991). Ebarb et al. (2016) also reported that WBSF was correlated with the CSA of all fibre types at day 14 of aging, but by day 21 post-mortem WBSF was only correlated with type IIX fibre CSA, suggesting that proteolysis may be most reduced by HGP in these fibres. Roy et al. (2015) associated HGP-induced beef toughness with increased hydroxy-pyridinoline crosslinks, although Ebarb et al. (2016) found that WBSF was not correlated with collagen characteristics at day 3 and day 12 post-mortem but insoluble collagen and total collagen were correlated with WBSF at day 21 post-mortem (Ebarb et al., 2016). Steers treated with ZH and HGP may have less or similar intramuscular collagen than non-treated steers (Kellermeier et al., 2009), the authors reported the reduced collagen because of dilution effect of muscle hypertrophy and reported no difference in WBSF and this may explain why some researchers found steers treated with HGP had mean WBSF values similar to control steers at day 14 or day 12 post-mortem (Igo et al., 2011) as total collagen and insoluble collagen become more important in affecting meat toughness during the later phases of aging as the influence of myofibrillar proteins decreases (Ebarb et al., 2016). Growth promotants, in general, are reported to increase the WBSF by increasing the CSA of muscle fibres rather than altering collagen structure (Ebarb et al., 2016). Ebarb et al. (2016) reported HGP increased CSA of type I and type IIX fibres by 20 % and 14 % respectively compared to control heifers, with the largest CSA observed in type IIX muscle

fibres from heifers that received both HGP and ZH, followed by HGP heifers and then control heifers. Gonzalez et al. (2007) and Fritsche et al. (2000) also reported increased CSA of muscle fibres with the use of a TBA and estradiol implant. β-AA supplementation also has been reported to increase CSA of muscle fibres (Kellermeier et al., 2009; Miller et al., 1988; Vestergaard et al., 1994; Phelps et al., 2014a).

Estrogenic (progesterone, estradiol benzoate), or androgenic (testosterone, testosterone proprionate, trenbolone acetate) hormonal growth promotants (Roebar et al., 2000) have separate mechanisms of increasing the feed efficiency. Estrogenic compounds boost the muscle and skeletal growth rates of an animal by encouraging the production and secretion of secondary hormones (hepatic somatotropin and insulin-like growth factor 1) which are attributed to the promotion of accumulation of muscle protein. Androgenic compounds stimulate protein synthesis by acting directly on muscle tissue and mitigate the rate of muscle protein degradation resulting in an increase in muscle mass (Reinhardt, 2007). The beef industry generally uses a combination of the two strategies as it is proven to be more efficient that a single strategy (Reinhardt, 2007). The probability of HGP to affect beef palatability may depend on the number of implants and whether the implants are estrogenic, androgenic or a combination of these two. Beef palatability may be reduced if cattle are subjected to two androgenic implants compared to a single implant or two estrogenic implants (NLSMB, 1995). Platter et al. (2001) found increased maturity scores in steers with a greater number of implants, but anabolic implants were not reported to have a direct effect on intramuscular fat (Smith et al., 2007).

The β-AA, ZH and RAC have been recorded in previous studies as inducing toughness of meat (Avendaño-Reyes et al., 2006; Scramlin et al., 2010; Arp et al., 2013; Garmyn and Miller, 2014). Bloomberg (2013) reported increased WBSF when cattle were fed with ZH. Leheska et al. (2009) reported that when ZH was supplemented in feed at 8.3 mg/kg (DM basis), there was an increase in left strip loin WBSF (3.3 vs. 4.0 kg, control versus treated). Overall, Hilton et al. (2009) and Mehaffey et al. (2009) suggested that the induced toughness is still usually less than the critical shear force value of 4.5 kg (Miller et al., 2001; Platter et al., 2003), so toughness induced by β-AA may not have a meaningful effect on consumer acceptance and palatability of beef. Within β-AA supplements, ZH is reported to have more adverse effect on WBSF than RAC (Garmyn et al., 2014), as the latter was not consistent in its effect in the previous studies, many of which suggested no effect of RAC on WBSF (Garmyn et al., 2014; Quinn et al., 2008). On the other hand, Boler et al. (2012) found that steaks from cattle fed RAC at four days post-mortem had 13% greater WBSF compared to controls, and Gruber et al. (2008) also reported increased WBSF for meat from steers fed RAC. RAC is also reported to show strong interaction with post-mortem aging, with lower WBSF than control steaks at day 21 post-mortem (Garmyn et al., 2014). O'Neill (2001) did not find any significant difference in WBSF values between beef from control steers and steers fed ZH but Rathman et al. (2009) reported that the control group (0 d) had lower WBSF values at all post mortem aging periods (7, 14, and 21 d) than cattle fed ZH. Literature suggests that the adverse effect of β-AA supplementation depends upon the dose used. Feed supplementation of RAC at 200 mg $steer^{-1}d^{-1}$ had no effect on WBSF but increases in WBSF were observed in muscles from carcasses of treated steers when used at a level of 300 mg $steer^{-1}d^{-1}$ (Arp et al., 2013).

The use of HGP (Ebarb et al., 2016; Ebarb et al., 2017; Boles et al., 2016) as well as β-AA feed supplementation increases cattle feed efficiency and increases hot carcass weight (Herscchler et al., 1995; Quinn et al., 2008). Growth hormones are reported to improve weight gain by 5 - 20%, feed efficiency by 5 - 12%, and lean meat growth by 15 - 25% (Kenney and Fallert, 1989). Ebarb et al. (2017) reported a more than 7.5 % increase in m. *longissimus* (LM) area from HGP or HGP and RAC treated steers. Griffin et al. (2009) found increased LM area with RAC supplementation whereas Bryant et al. (2010) reported no difference in LM area due to RAC. Growth promotants have been reported to increase lean muscle yield (Schmidt and Olsan, 2007) by directing nutrients towards growth of lean muscle instead of adipose tissue (Duckett et al., 1999), which substantiates the decrease in marbling and subcutaneous fat observed in the carcasses of cattle supplemented with the β-AA clenbuterol (Strydom et al., 2009) and ZH (Arp et al., 2014; Ebarb et al., 2016). Other work suggests no effect of RAC or ZH on intramuscular fat content (Talton et al., 2014; Strydom et al., 2009; Garmyn et al., 2014) and generally, in the literature, β-AA are not reported to affect fat content (Cook et al., 2000; DeHaan et al., 1990). The effect of β-AA on carcass fat may differ due to the type of β-AA used and the muscle type studied. Strydom et al. (2009) reported steers treated with RAC had the same intramuscular fat as control steers in the m. *semitendinosus* but reduced fat in the LM while ZH treated steers tended towards reduced marbling scores and 12th rib subcutaneous fat irrespective of muscle type (Alam et al., 2009; Strydom et al., 2009). Similarly, Avendaño-Reyes et al. (2006) reported no difference in 12th subcutaneous rib fat between RAC and the control steers but a tendency toward less 12th subcutaneous rib fat in ZH steers than the control steers. Arp et al. (2014) reported a decreased marbling score between the 12th and 13th ribs when RAC was used at 300 mg steer^{-1} d^{-1} but no effect was observed at 200 mg/steer/day. Cimaterol was also reported to reduce the

deposition of adipose tissue (Chikhou et al., 1993b). Garmyn et al. (2014) reported increased muscle protein in ZH fed steers than those from control steers or RAC fed steers, which were similar.

Little research appears to be available assessing the combined effect of β-AA and HGP but the available literature suggests that β-AA have a synergistic effect with HGP on WBSF, with increased WBSF in COMBO (steroids + ZH) steaks relative to that from HGP only steers. Ebarb et al. (2017) reported that meat from the COMBO treatment was significantly tougher than the control group and the meat from the HGP treated group. Kellermeier et al. (2009) reported that when ZH is fed to cattle that received an estrogen + TBA terminal implant, ZH potentially increased LM WBSF because of the profound muscle hypertrophy it induces, with tenderness reduced even after a post mortem aging period of 21 days.

With regard to meat quality characteristics other than WBSF and toughness, cooking loss was found not to be affected by β-AA supplementation (Hilton et al., 2009; Kellermeier et al., 2009; Garmyn et al., 2010) although Garmyn et al. (2014) reported an increased cooking loss in ZH supplemented steers but not in RAC treated steers. β-AA treatment may not have a direct effect on drip loss but showed an interaction with a day of aging for drip loss, with drip loss first decreasing and then increasing with days of aging (Avendaño-Reyes et al., 2006). The effect on water holding capacity (WHC) of meat may vary with each β-AA, with Avendaño-Reyes et al., (2006) observing WHC decreasing from day 5 post-mortem in RAC treated steers in a manner similar to the control group while ZH treated steers showed an opposite effect with WHC increasing with post-mortem aging.

Generally, RFI is not recorded to have any effect on beef toughness (McDonagh et al., 2001; Baker et al., 2006; Reis et al., 2015 and Ahola et al., 2011). Bulle et al. (2007) suggested that RFI efficient animals may have low protein turnover and that calpastatin activity may increase in muscle after 4-5 generations of selective breeding for low RFI animals (McDonagh et al., 2001). Although RFI has not yet been associated with beef toughness, it may have an additive effect on beef toughness if cattle selectively bred for low RFI were treated with growth promotants (HGP/ β-AA). This possibility has yet to be investigated, but warrants consideration.

Other pre-slaughter factors that can affect meat quality include transport conditions and pre-slaughter stress. Heat stress during transportation increases animal restlessness, heart rate, and rectal temperature. This stress may result in the rapid loss of muscle glycogen, which is required in the conversion of muscle into meat, and in beef it may lead to dark, firm and dry (DFD) meat (pH > 5.9) (Young et al., 2004; Gregory, 2010; Gajana et al., 2013). Mixing unfamiliar cattle before slaughter is a critical source of stress and leads to increased plasma creatine phospho-kinase (CPK) activity and free fatty acid (FFA) concentration and a decrease in plasma lactate and depletion of liver and muscle glycogen resulting in dark cutting meat (Ferguson et al., 2001; Warris, 1984). If unfamiliar herds must be mixed, then a resting period of at least 48 hours with food is required before slaughter to prevent dark cutting beef (Warris, 1984). Pre-slaughter supplements like sugar and electrolytes have been reported to compensate for transport stress by reducing glycogenolysis and thus reducing the probability of dark cutting (Schaefer et al., 1997).

Post-slaughter Factors

Apart from the intrinsic animal factors (genetics, breed, sex, and age), conditions that prevail the 24-48 hours before and after slaughter can significantly impact beef palatability (Ferguson et al., 2001). Post-slaughter factors such as product storage time and temperature also affect the quality of meat (Locker and Hagyard, 1963). Chilling temperature is a critical factor affecting meat quality as if pre-rigor muscles are exposed to cold (less than 10 °C in less than 10 hours post-mortem), they will enter rigor in a contracted state, a condition called cold shortening. This contracture occurs due to a thirty- to forty-fold increase in the calcium ion concentration in the myofibrillar region in response to a rapid decrease in temperature before all the muscle glycogen has been converted into lactic acid (Davey et al., 1967). Minimum sarcomere shortening takes place when the meat enters rigor between 14–19° C (Locker and Hagyard, 1963). Electrical stimulation is an efficient technique to prevent cold shortening by exhausting adenosine triphosphate (ATP) concentrations quickly during the early post-mortem period. A voltage of 3600 V for 1-2 min after dressing reduces the time of onset of rigor up to 5 hours, which will ensure that the meat will be warm when entering rigor thus preventing cold shortening (Davey et al., 1976). However, higher post-mortem temperature is associated with increased rate of pH decline (Bruce and Ball, 1990) due to increased rate of glycolysis (Janz et al., 2001) and may lead to heat shortening if the pH falls below 6.0 while the muscle temperature is still above 35 ^{o} C. Rates of pH decline can increase with carcass electrical stimulation by up to 2 fold. This rate and magnitude of pH decline is dependent on the duration of stimulation, voltage, current, frequency and wave form (Chrystall and Devine, 1978).

Increased beef toughness due to the use of growth promotants may be alleviated by proteolysis during aging. HGP treated cattle usually reach similar levels of toughness to that of beef from cattle not treated with HGP by 14-21 days post-mortem (Ebarb et al., 2016). Post-mortem aging appears to mitigate the adverse effect of both HGP and β-AA (Platter et al., 2003; Ebarb et al., 2016), however, there are discrepancies regarding the minimum aging period required to completely eliminate toughness induced by growth promotants. Ebarb et al. (2016) reported no difference in the toughness of m. *longissimus lumborum* steaks from HGP and control heifers by day 21 post-mortem, while Platter et al. (2007) observed higher mean WBSF in the same muscle from the carcasses of implanted heifers at day 21 post mortem. In general, the literature suggests that 14-28 days of aging will reduce the toughness induced by growth promotant use in cattle (Schneider et al., 2007; Boles et al., 2009).

As beef is heterogeneous in nature, and although its quality characteristics may be influenced by many pre-slaughter and post-slaughter factors discussed, the variability in meat quality is the main concern for the consumers (Leroy et al., 2003). Beef consumers are demanding quality that meets their expectations, particularly for beef, and this may require development of a beef quality guarantee system in the Canadian meat industry (Verbeke et al., 2010). To provide this guarantee, the quality of meat and its subsequent products should be at the very least measurable and ideally predictable.

Methods available to measure meat quality characteristics:

Methods such as chemical analysis, instrumental measurements and sensory analysis are used to evaluate several chemical, technological and sensory characteristics of meat. Sensory panel evaluation has been traditionally used to determine beef eating quality. Descriptive sensory

analysis requires a trained panel which ideally should consist of both men and women from different age groups, and panel members should have the ability to discriminate and describe the product based on the quality factor being assessed. Sort of panel needs preliminary training to express proportionality within the different sensory test methods (Meilgaard et al., 1999). The reliability of sensory analysis depends largely on the preliminary training of the panel before sensory profiling as it allows for the development of a relationship between descriptive sensory and consumer preference measurements (O'Sullivan & Kerry, 2009). Although extremely effective at describing and estimating meat quality characteristics, sensory panel analysis is a costly and time-consuming method (Vidal et al., 2014). As a result, objective instrumentation is often used to reduce the cost of assessment and speed the acquisition of results.

Chemical procedures are traditional and widely acceptable but time-consuming and involve the use of hazardous solvents and chemicals. The official method of the Association of Official Agricultural Chemists (AOAC) for fat analysis involves solvent extraction using petroleum ether of a dried product. The main disadvantage of the conventional Soxhlet extraction for solid sample preparation is the protracted time required for the extraction and the expensive solvents, the disposal of which may lead to environmental contamination (Luque de Castro et al., 2010). Although a rapid method for fat extraction based on AOAC method 960.39 is used by the Foss Soxtec™ 2050 (Foss Analytical, Hilleroed, Denmark) which provides results in 3 hours, it is destructive, involves hours of sample preparation and is not suitable for online application (Salvin et al., 1955; Liu et al., 2004). Further, extraction methods require solvents that are costly, hazardous, and often present disposal and storage challenges (Leffler et al., 2008).

Instrumental colour analysis of meat is often performed using colorimeters and spectrophotometers that employ the Hunter, CIE or tri-stimulus colour systems. Further, there are different illuminants (A, C, D65, and Ultraume), aperture sizes (0.64-3.2 cm) and observers angles (2^o and 10^o) available, although no differences have been reported in colour measures between different illuminants and the degree angle of the observer (Gracia-Esteben et al., 2003). These spectrophotometers have a small measurement area which requires analysers to take multiple readings to cover the colour variation in a muscle face. Secondary methods like digital imaging and NIR spectroscopy may have advantages over the traditional instruments in their ability to provide comprehensive coverage of the surface variation. Computer vision systems use analysis of digital images, and visible (VIS) light bands at 440, 475, 535, and 635 nm are correlated with deoxymyoglobin, metmyoglobin, oxymyoglobin, and sulfmyoglobin concentrations, respectively (Liu et al., 2003).

There have been many attempts to predict L*, a*, b* values using combined VIS and NIR spectroscopy. Andres et al. (2008) reported a prediction R^2 value between VIS/NIR spectra and CIE colour data of 0.82 for L*, 0.35 for a* and 0.51 for b* values. Similarly, Leroy et al. (2003) and Prieto et al. (2008a) reported high prediction R^2 values of 0.64-0.85 and 0.59/0.87 for L* respectively, 0.19-0.49 and 0.008/0.71 for a*, 0.44-0.75 and 0.35/0.90 for b*. Most of the previous work reported low R^2 values for a* compared to L* and b*, and this could be due to few wavelengths (580–630 nm) being attributed to the colour red (Prieto et al., 2009). On the contrary, Liu et al. (2003) reported an R^2 of 0.9, 0.78, and 0.55 for a*, b*and L*using spectroscopy incorporating both visible (400-700 nm) and NIR (700-1080 nm) light. Integration of both visible and NIR regions in spectroscopy and a large number of scans appears to

compensate for heterogeneity within the sample and may have enabled Liu et al. (2003) to achieve a high R^2 value for a*.

There are several methods used to assess the toughness of meat, and each method has its own advantages and disadvantages. The most common methods are sensory analysis, Warner-Bratzler Shear Force (WBSF), sarcomere length, computer image analysis, and infrared spectroscopy. Traditionally, the most common test to detect the texture of food products uses a universal testing machine (UTM). Since the 1990s, materials testing machines have been designed specifically to suit the food market with a range of appropriate fixtures to conduct specific tests. The most popular test to analyse the texture of meat products is the shear test, which has many forms: the Warner-Bratzler Shear Force (WBSF); the slice shear force (SSF) test; the Kramer Shear (KS); and the compression test (Texture Profile Analysis) (Smith et al., 1988; Shackelford et al., 2004).

The standard Warner Bratzler shear force analysis involves measurement of cooked meat tenderness using a Warner Bratzler blade that is consistent to the following specifications: a) shearing blade thickness of 1.016 mm; b) a V-shaped cutting blade with a 60 degree angle; c) the cutting edge beveled to a half-round; d) the corner of the V is rounded to a quarter-round of a 2.363 mm diameter circle; e) the space providing the gap for the cutting blade to slide through should be 1.245mm wide; f) the cooked meat samples should be round cores 1.27 cm in diameter removed parallel to the longitudinal orientation of the muscle fibres; and g) the cores should be sheared once at the center, perpendicular to the fibres to avoid the hard surface of the cooked sample (Bratzler, 1932, 1949; AMSA, 1995; Wheeler et al, 1995). The maximum or peak force

is termed the Warner Bratzler shear force. The principle of the method is based on the simulation of shearing food products in the mouth with teeth. Wheeler et al. (1997) emphasized the importance of following a standard protocol for WBSF measurement as differences in protocol and instrument variation can lead to poor repeatability of the method. The protocol should include the width of the blades and the position of the V-notch, the test speed, sample preparation and sample presentation as Warner Bratzler shear force measurement is influenced by sample shape and size, muscle fibres direction, distribution and amount of connective tissue and intramuscular fat, sample temperature, and test speed (Lyon and Lyon., 1998). The sample should be sheared at right angles to the muscle fibre axis. The characteristic to be measured from the force deformation curve is the peak force (the maximum recorded shear value).

The Kramer shear accessory consists of a cell into which a product is filled and a five or ten-bladed compression head. The use of multiple blades rather than a single shear blade compensates for the heterogeneity present within the sample by averaging the forces required to shear the sample at multiple positions rather than a specific position.

Texture profile analysis (TPA), which is a double compression test, has also been used to assess the textural quality of meat (Caine et al., 2003; Ruiz de Huidobro et al., 2005). TPA consists of a double compression test during which several textural properties of a product are analysed based on the deformation of the product in the first compression cycle and in the second compression cycle. TPA has an advantage of assessing several properties of the texture of the product and may be of great significance in describing meat texture as meat texture may rarely be described by a single textural property as it is an amalgamation of multiple textural characteristics that

culminate in a total eating experience. TPA can quantify these multiple textural characteristics in just one experiment. Texture characteristics that can be described using TPA include hardness, cohesiveness, adhesiveness, springiness, resilience and chewiness or gumminess. The following measurements are considered to be most important in the assessment of meat quality using TPA (Bourne, 1978): maximum force (N) required to compress the sample to a target distance or strain value, which indicates toughness; springiness (m), which is the ability of the sample to recover its original form after being forcibly deformed; chewiness; and finally cohesiveness, which is the extent to which the sample could be deformed before rupturing. Toughness and chewability (toughness x springiness x cohesiveness) appear to relate most to the desirability of meat (Guzek, 2013; Ruiz de Huidobro et al., 2001). Ruiz de Huidobro et al. (2005) found TPA to be a better instrumental texture analysis method than WBSF to determine cooked meat texture given that analyses were done on raw beef as the toughness recorded by TPA method was better correlated with sensory tenderness than WBSF and the WBSF method had a high coefficient of variation. However, the authors suggested the use of WBSF to determine meat toughness if the analyses were done on cooked beef. It can also be argued that shear force determination has an advantage over TPA as the former simulates the first action used by consumers to perceive meat texture which is to cut through the muscle fibers (Lyon and Lyon, 1998).

Although WBSF is globally accepted and is the most popular method used to predict the toughness of meat, it has its own limitations. This method involves sample destruction, requires trained personnel to perform the test and to interpret the results, and sample preparation is prone to lack of homogeneity due to human error. Also, the steak used for WBSF analysis cannot be used for human consumption and so it is lost as a saleable product, which is one of the major problems associated with using WBSF on an industrial scale (Liu, 2003). To be used on an

industrial scale, a method should be able to examine a large number of samples in a short span of time and provide the results immediately (Caine et al., 2003). The WBSF method does not meet the requirement of the meat industry for a fast and non-invasive test.

Visible and near-infrared spectroscopy

NIR spectroscopy has been effectively used by the dairy industry to determine lactose, fat, and protein. Also, the grain and livestock feed industry are taking full advantage of IR spectroscopy owing to its fast and accurate nature (Shenk et al., 1979; Bjarno, 1982). It has also been used in the meat industry, mostly to predict chemical composition through quantitation of fat and protein content (Tøgerson et al., 1999; Hildrum et al., 1999; Denoyelle et al., 1996). The NIR spectral band stretches from 780 to 2500 nm in the electromagnetic spectrum and provides structural information about the vibration behavior of organic bonds including C-H, O-H, N-H, and C-O bonds. These bonds, when illuminated by NIR light, may either stretch or bend by absorbing light at specific wavelengths, and this record is translated into an absorption spectrum within the spectrometer (Cen et al., 2007). As energy is absorbed, peaks are produced in the NIR spectrum. The intensity of the peaks produced in the NIR spectrum depends on the change in the dipole moment of a bond undergoing vibration and on the number of specific bonds present. Apart from the peaks providing fundamental vibrations, most other peaks are 1^{st}, 2^{nd} or 3^{rd} overtones of a combination of initial vibrations (Blanco and Villarroya, 2002). The absorption intensity decreases when the overtone increases. As an example, the absorption bands for the O-H bond specifically occur at 948, 1448 and about 1940 nm (Shenk et al., 1992), with peaks at 948 nm and 1448 nm being due to the second and first stretching overtone of the O-H bond, respectively (Cozzolino and Murray, 2002; Ortiz-Somovilla et al., 2007; Morsy and Sun, 2013). The

absorption bands of the N-H bond occur from 1460-1570 nm and 2000-2180 nm (Murray and Williams, 1987; Shenk et al., 1992). The absorption of C-H bond is observed from 1100-1400 nm, around 1700, and from 2200 – 2400 nm, with specific absorption at 920, 1200 and 1716 and 1758 nm because of the 3rd, 2nd and 1st overtone from the C-H bond, respectively, while the combination tones are observed from 2100-2400 nm (Park et al., 2001; Morsy and Sun, 2013).

Being a popular and successful quality assessment technology in the feed and grain industry, NIR spectroscopy has attracted many researchers to explore its application in the meat industry. A number of studies have been published to assess the potential of NIR to predict the chemical composition of meat. Most have confirmed the ability of NIR spectroscopy to predict intramuscular fat (IMF), crude protein (CP), and moisture (Togersen et al., 2003; Prevolnik et al., 2005; Prieto et al., 2006). While there are studies claiming to predict IMF in meat with the best accuracy by NIR spectroscopy (Anderson and Walker, 2003; Togersen et al., 2003; De Marchi et al., 2007) there are some which were unsuccessful (Rodbotten et al., 2000). Poor accuracy of prediction using NIR may arise from poor calibration, as good calibration depends on the accuracy of the reference data and the variability present in the sample set used for calibration. Also, sample preparation has a major role in the success of NIR analysis. In the prediction of chemical components, minced or ground samples are likely to be predicted with better accuracy than intact tissue because mincing improves product homogeneity. Also, in intact meat, the presence of myofibrils could affect light reflection and scattering. Myofibrils themselves can act as optical fibres and can conduct the light along their length by a series of internal reflection (Prieto et al., 2009)

There have been many attempts to explore the ability of NIR to determine technological characteristics of meat like pH, colour – L*, a* and b* value, water holding capacity and tenderness. There is no satisfactory algorithm established for pH value but Andres et al. (2008) was able to achieve an R^2 value of 0.97 between actual and predicted pH data. For the prediction of L*, a* and b*, it was observed that a combination of VIS and NIR led to increased R^2 prediction values compared to the results of NIR alone (Cozzolino et al., 2003). It may be because the a* value is dependent on myoglobin concentration, and myoglobin is associated with a wavelength range from 425 nm to 550 nm, well into the visible wavelength range (Ripoll et al., 2008). Andres et al. (2008), Cozolinno et al. (2003) and Liu et al. (2003) used combined VIS/NIR regions while Leroy et al. (2003) and Prieto et al. (2008a) used only the NIR region to obtain prediction R^2 values greater than 0.85 for L*, a* and/or b*. Researchers have found that NIR was not able to predict the water holding capacity in the meat, although Ripoll et al. (2008) achieved an R^2 value of 0.71, while others reported R^2 values ranging from 0.001 to 0.58 (Andres et al., 2008; Leroy et al., 2003; Prieto et al., 2008a; De Marchi et al., 2007).

Most authors infer a limited application of NIR to predict toughness of beef (Table 1.1). Of the authors listed in Table 1.1, Meulemans et al. (2003), De Marchi et al. (2007), Prieto et al. (2008) and Ripoll et al. (2008) conducted experiments on minced or homogenized meat. Although Ripoll et al. (2008) obtained a prediction R^2 value of 0.74, others were not able to achieve an R^2 of more than 0.2. Barlocco et al. (2006) conducted a NIR analysis of both minced and intact meat from the same samples, and obtained an R^2 value of 0.38 for the homogenized meat and an R^2 value of 0.64 for the intact meat. It can be inferred that homogenized/minced meat is not suitable for toughness measurement as in minced meat the structure of the muscle and fibre direction is

destroyed. However, Ripoll et al. (2008) reported an R^2 value of 0.74 by collecting spectra of minced meat. Ripoll et al. (2008) explained that there is a correlation between WBSF and moisture, arising from a very strong inverse relation between moisture and fat and between

Table1.1: Prediction of WBSF in beef by NIR spectroscopy. R^2: Coefficient of determination of calibration.

Author	Meat	R^2
Byrne et al. (1998)	Beef	0.37-0.67
Chan et al. (2002)	Pork	0.17
Geesink et al. (2003)	Pork	0.2
Leroy et al. (2003)	Beef	0.12-0.14
Liu et al. (2003)	Beef	0.17-0.72
Meulemans et al. (2003)	Pork	Not reliable
Liu et al. (2004)	Poultry	0.45/0.53
De Marchi et al. (2007)	Beef	0.08/0.2
Prieto et al. (2008)	Beef	0.17/0.45
Venel et al. (2001)	Beef	0.58
Rodbotten et al. (2001)	Beef	0.36-0.69
Park et al. (1998)	Beef	0.67
Andres et al. (2008)	Beef	0.65
Meullenet et al. (2004)	Poultry	0.3-0.9
Ripoll et al. (2008)	Beef	0.74
Borlocco et al. (2006)	Pork	0.64/0.38

WBSF and WHC, and therefore minced meat could give significant information about moisture, fat, and WHC, hence giving higher R^2 value for WBSF. Also, Ripoll et al. (2008) used a wide range of WBSF reference data (coefficient of variation = 31%) which explains the high accuracy of their predictive models. On the other hand, Leroy et al. (2003) used intact samples but randomly chose the animals without selection for a range of toughness. This may explain the very low R^2 value of 0.14 obtained by these researchers.

Researchers have had some success in differentiating tough and tender beef using visible light (Liu et al, 2003), but effectively discerning differences in toughness of beef that is not extremely tough or tender have been elusive. Peng and Wang (2015) found that VIS/NIR wavelengths, specifically 437, 549, 646, 700, 718, 776, and 849 nm, could predict tenderness with 88% accuracy. Wu et al. (2012) also found that *longissimus* muscle toughness could be predicted with 91% accuracy using wavelengths similar to those of Peng and Wang (2015). Strong correlations between VIS light wavelengths and colour may indicate that intramuscular fat and pH may be the operative muscle characteristics in these studies, as both can affect colour reflectance through oxidation of myoglobin (Suman and Joseph 2013) and electron transport chain function (Bendall and Taylor 1972).

Prieto et al. (2008) used the quality mark "Valles del Esla" to select young steers and oxen and reported that the homogeneity achieved by the quality mark was the main reason for a low R^2 value. However, the use of different slices of meat for collecting spectra and for reference WBSF analysis could also be the reason for obtaining a low R^2. Venel et al. (2001) observed that the calibrations for toughness and other quality attributes of beef were improved when the sample set

was segregated according to sex, animal grade, ultimate pH or day of carcass fabrication. Clearly, selection of animals plays an important role in developing a successful calibration. The calibration sample set should have substantial variation and include extreme toughness values. A robust calibration model is dependent upon the range of reference values and could be increased by using a larger number of samples (Meulemans et al., 2003).

The reference method plays a vital role in building a satisfactory calibration. Chan et al. (2002) found a large variation between the WBSF values of different cores from the same steak. WBSF involves preparing cores across the surface of a steak (Bratzler, 1932, 1949; AMSA, 1995; Wheeler et al., 1995), and it is difficult to obtain identical cores from a cooked steak (Honikel, 1998). Barlocco et al. (2006) reported the limited application of NIR to predict WBSF was due to large standard deviations involved in the reference method.

Development of a successful calibration model also depends upon chemometrics. Generally, before any kind of pre-treatments or regression modeling, principal component analysis (PCA) is performed on data sets to detect any potential grouping of samples and to identify any outliers in the data. These outliers are then removed from the sample set before any further analysis (Venel et al., 2001; Borlocco et al., 2006; Liu et al., 2004). Often the collected spectral data have a significant amount of background information in addition to sample information and is too noisy to process. In such cases pre-processing techniques are used to reduce random variability in the spectral data and promote linear relationships between explanatory variables and spectral data. Popular pre-processing methods include smoothing, 1st and 2nd order derivatization, multiplicative scatter correction (MSC), standard normal variate transformation (SNV), Fourier

transformation (FT), and orthogonal signal correction (OSC) (Cen et al., 2007; Gautam et al., 2015; Luypaert et al., 2004). In almost every case, smoothing of the data occurred before it was modeled. Several types of smoothing can be employed and include moving average, Gaussian filter, median filter and Savitzkye Golay (SG) smoothing. Care must be taken while selecting the width of the smoothing window to remove unwanted noise from the spectral data without loss of what may be important sample information. SG smoothing is the most popular type of smoothing (Gorry, 1990) and has been used by a number of authors. Barlocco et al. (2006) used 19-point SG second order filtering option, and Liu et. al. (2004) used 11-point SG smoothing.

Transformation of spectra by first or second order derivatives is used to correct for baseline shift and to separate superimposing peaks (Luypaert et al., 2004). The first order derivative provides the rate of change of absorption with wavelength and removes the baseline (Rinnan et al., 2009). This results in a peak where there is a maximum slope in the original spectrum and results in zero where the original spectra had a peak. The second order derivative is the differential of the first order derivative and gives the rate of change of the slope of the absorbance. By transforming data into first and second order derivatives, peaks and valleys not obvious in the original spectra become clear (Cen et. al., 2007). The treated spectra may look more complex than the original spectra (Geesink et al., 2003; Rinnan et al., 2009), so derivative pre-treatment is generally accompanied by smoothing to reduce the noise and complexity of the spectrum and overlapping peaks (Borlocco et al., 2006; Park et al., 1998; Ripoll et al., 2008).

SNV removes the baseline shifts of the spectra and corrects for both multiplicative and additive scatter effects by removing the multiplicative interferences of particle size, scattering effect, and

the change of light distance (Andres et al., 2008, Barnes et al., 1989; Isaksson et al., 1988; Rodbotten et al., 2000). Rodbotten et al. (2000) used MSC to remove multiplicative and additive scatter effects. Orthogonal signal correlation (OSC) (Wold et al., 1998) corrects for instrumental drift, bias, and scattering effect in NIR spectra. OSC prevents the loss of essential information by removing random variation that is orthogonal to the property of interest. Some authors have indicated that OSC is a better pre-processing technique than others (Luypaert et al., 2004; Pizarro et al., 2004; Roggo et al., 2003). Usually partial least squares (PLS) regression (Lindberg et al., 1983; Leroy et al., 2003; Prieto et al., 2008) or multiple linear regression (MLR) method (Hruschka, 1987) are used for chemometric analysis of pre-treated spectral data.

NIR spectroscopy seems to be a very promising technology in the field of food analysis. It is fast, repeatable, easy to use, can perform multiple component analysis at the same time and most importantly is non-invasive. This means that it is possible to analyze samples that are planned to be sold afterward. Although there are industries like the cattle feed and grain industries in which researchers have performed enough work to explore the full potential of near-infrared spectroscopy, there is a requirement for further research in the meat industry especially to predict toughness using NIR spectroscopy (Barlocco et al., 2006; Ripoll et al., 2008). By controlling the factors influencing the spectral data and by collecting high precision reference data, NIR spectroscopy may be able to predict beef toughness accurately. Although the disadvantage of this method is that it requires time-consuming and labor-intensive calibration, once calibration has been established it needs to be updated only when there is a change or modification in the characteristics of the sample. NIR has the potential to predict meat toughness (Andres et al., 2008) but further work is required to improve NIR before its application in the meat industry.

As different growth promotants (HGP, β-AA, RFI) are currently an integral part of cattle production, it is important to include their effect on meat quality in the assessment of NIR spectroscopy as a predictive technology. Also, the effects of selection for RFI need to be included, as RFI may become an important genetic selection criterion in the Canadian beef industry. During this review, it also became apparent that the effects of steroids, β-AA and RFI are understood independently but there is very limited understanding of the effect of the combination of these treatments on beef quality and their interaction with post-mortem aging.

The objective of the first experiment in this thesis therefore was to evaluate the effect of HGP, β-AA, RFI and post-mortem aging and the interaction of these treatments on the quality characteristics of bovine m. *longissimus thoracis* (LT). The results of this experiment will answer if the different growth-promoting strategies, specifically HGP, β-AA and RFI, have an accentuated effect on meat quality when being used in combination with each other. Understanding this interaction will, in turn, help producers to plan appropriate growth promotant strategies to maximize their economic advantage without compromising meat quality, and also indicate if there is substantial variation in the meat quality characteristics of beef from cattle arising from these production practices.

The objective of the second experiment was to determine if NIR spectroscopy could be used to predict the meat quality characteristics measured in the population of cattle from the first experiment.

To achieve both of these objectives, the following hypotheses were to be tested:

1. That HGP and cattle with low RFI produce beef with mean WBSF values greater than those of cattle receiving RAC or no growth promoting technology;
2. That RAC supplementation increases mean WBSF relative to cattle that do not receive RAC;
3. That toughness due to growth promotants may be alleviated with post mortem aging; and
4. That NIR spectra can be grouped into algorithms that predict meat quality characteristics with R^2 values greater than 0.80.

To test these hypotheses, experimentation was undertaken to achieve the following objectives:

1. To determine if WBSF and other meat quality measurements were affected by HGP implantation, supplementation of cattle with RAC, selection for RFI performance, and by post mortem aging for 12 days;
2. To relate NIR spectra from 900-1650 nm to WBSF, colour, pH and drip loss and determine the R^2 for the regressions between NIR and the meat quality characteristics.

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Chapter 2: Effect of hormonal growth promotants, β-adrenergic agonists feed supplementation and residual feed intake on the quality characteristics of bovine m. *longissimus thoracis*

2.1 Introduction:

Growth promotants are profitable tools for cattle producers to improve feed efficiency and animal performance (Dikeman, 2007; Duckett and Pratt, 2014). Cattle production costs may increase by $18/head if producers choose not to use hormonal growth promotants and feedlot production costs have been shown to increase by 10% if no growth promotants (implants, ionophores or beta agonists) are used (Lawrence and Ibarburu, 2007). The two types of growth promotants (GPs) approved for use in cattle are steroids or hormonal growth promotants (HGP), and β-adrenergic agonists (β-AA). HGP are pharmaceutical drugs that function similar to an endogenous growth hormone in an animal. They are implanted under the skin behind the ear from where they slowly release the drug into the blood of an animal.

β-AA are synthetic compounds which, once absorbed through the digestive tract and into the blood, bind to β-adrenergic receptors on the cell surface and stimulate those receptors to promote muscle growth in an animal (Mersmannn et al., 1998). β-AA are added to the feed during the last four to six weeks of the finishing period of cattle. Type II muscle fibers are most sensitive to β-AA and this leads to an increased muscle fiber cross-sectional area that, in turn, is responsible for the increase in muscle mass (Miller et al., 1988; Smith et al., 1995; Johnson, 2014). β-AA reportedly decreases the proportion of trimmed fat and increases the proportion of lean meat as well (Moloney et al., 1990). Well-documented β-AA include ractopamine hydrochloride (RAC), zilpaterol hydrochloride (ZH), clenbuterol, L-644,969, and cimaterol, all of which were reported

to increase carcass weight gain and feed efficiency in beef cattle (Lean et al., 2014; Johnson et al., 2014). Out of these, in Canada and the United States, RAC and ZH are the only β-AA approved and labeled for use as a feed additive for cattle by the Food and Drug Administration (FDA, 2003; FDA, 2006) and the Veterinary Drug Directorate of Canada.

Apart from increasing the efficiency of cattle by increasing muscle gain per kg of feed, HGP and β-AA use is associated with reduced calpain activity (Strydom et al., 2009), increased muscle fiber cross-sectional area (Ebarb et al., 2016) and increased hydroxyl-pyridinoline crosslinks (Roy et al., 2015), all of which have been associated with increased beef toughness (Crouse et al., 1991; Weston et al., 2002; Bhat et al., 2018). Increased beef toughness due to HGP may be alleviated with proteolysis during post mortem aging, with beef usually reaching the same levels of WBSF values as beef from cattle not treated with HGP by 14-21 days post-mortem (Ebarb et al., 2016). Combined treatment of cattle with both HGP implantation and β-AA supplementation may lead to even tougher meat than that observed in cattle to which either steroid or β-AA are applied alone (Ebarb et al., 2017).

As HGP and β-AA are becoming less acceptable to the beef consuming public (Dickinson and Bailey, 2002; Lusk et al., 2002; Alfnes, 2004; Alfnes and Rickertsen, 2003), improvement of cattle feed efficiency through selection for residual feed intake (RFI) has been investigated. Selection for residual feed intake (RFI) improves the feed efficiency of cattle without increasing mature size (Archer et al., 1998; Schenkel et al., 2004), and its use in herd selection criteria may not affect beef toughness (Baker et al., 2006). Whether the effects of RFI, HGP and β-AA are cumulative and how RFI interacts with HGP and β-AA does not appear to have been explored in

the literature, although the effects of hormonal growth implants on the expression of genes related to residual feed intake have been investigated (Al-Husseini et al., 2014). It is important that RFI selection does not interact with other growth promotants to adversely affect meat toughness and other meat quality characteristics. In previous research (Roeber et al., 2000; Avendano-Reyes et al., 2006; Ahola et al., 2011), the effect of steroids, β-AA and RFI were discussed independently but there is limited and contradictory understanding about the effect of the combination of these treatments on beef quality and their interactions with post-mortem aging. The objective of this paper is to evaluate the effects of HGP, β-AA, RFI and post-mortem aging and the interaction of these treatments on the quality characteristics of bovine m. *longissimus thoracis* (LT).

2.2 Material and methods

2.2.1 Animal Care: The animals used in this study were managed according to the University of Alberta's animal care and use program which meets the national standards sets by Canadian Council of Animal Care (CCAC). The study was reviewed by a University of Alberta animal care and use committee prior to its execution (Animal Use Protocol number AUP00001801).

2.2.2 Experimental design: Forty-eight (48) Angus crossbred beef steers were randomly selected from the University of Alberta beef herd and were stratified based on body weight and then randomized to treatment to minimize experimental error due to biological variation. Steers randomly available from University of Alberta's two beef herds, 21 control (RFI = 0.59 ± 0.67) and 27 RFI efficient (RFI = 0.16 ± 0.58), were selected and stratified for weight. Cattle from

each RFI group were randomly assigned to one of four treatments, specifically, steroid hormone growth promotants (HGP) only, β –adrenergic agonist (β-AA) only, both HGP and β-AA (COMBO), and controls which received neither HGP nor β-AA (CONT). The experiment was designed as a 2 (low, high RFI) x 2 (no, yes steroids) x 2 (no, yes β-AA) factorial. Steers were weaned at approximately 6 months old and were fed on a high forage (hay) diet for 120 days, after which they were stratified by weaning weight and randomly assigned to HGP and β-AA treatments. At a mean age of 320 days (10-11 months) and mean weight of 382.84 ± 19.74 kg, they were implanted with Component E-S (200 mg progesterone and 20 mg estradiol benzoate with 29 mg tylosin tartrate; Elanco Animal Health, Eli Lily Co., Greenfield, Indiana). Steers were then gradually alimented on to a high-grain finishing diet which consisted of 27% barley silage, 61% barley, 8% canola meal, and 4% mineral and rumensin pre-mix. Eighty (80) days after the first implant they were implanted with Component TE-S (120 mg trenbolone acetate (TBA) and 24 mg estradiol; Elanco Animal Health, Eli Lily Co., Greenfield, Indiana). Cattle received the same diet except for the group β-AA and COMBO, which were supplemented with RAC at manufacturer's recommended levels of 200mg/head/day for the last 28 days before slaughter. The minimum difference between the second implant and initiating RAC supplementation was 65 days as Johnson (2014) suggested that implantation of beef cattle 60 to 90 days before feeding β-AA should have additive effects on lean tissue deposition.

Cattle were fed at the University of Alberta Roy Berg Kinsella Research Ranch, Kinsella, Alberta, until transported to and slaughtered at a provincially inspected abattoir in Alberta. Steers were stratified by weight from heaviest to lightest prior to initiation of RAC treatment into

blocks with one animal per treatment in each block for slaughter. Cattle were slaughtered over 6 weeks with 8 cattle killed per week in a randomized complete block design.

Following slaughter, carcasses were split into halves and intramuscular pH and temperatures were measured after 1 and 3 hours post-exsanguination by making three punctures on the right side of the carcass at the 12^{th} – 13^{th} rib with a puncture electrode (Cat No. 655-500-30, FC210B, Canada-wide Scientific, Ottawa, ON) attached to an Accumet AP71 pH/temperature meter (Thermo Fisher Scientific, USA). The three pH values obtained at each time for each carcass were averaged, and the mean pH was used for statistical analysis. Following chilling of the carcasses for 48 hours, personnel trained in beef grading recorded from each carcass at the 12^{th} and 13^{th} rib interface the muscle score, grade marbling score, grade fat depth, and colour. Carcasses were then fabricated and the LT (rib eye) muscles were excised on the right side of the carcass. The LT muscles were packaged under vacuum in polypropylene bags and transported in ice-packed coolers to the University of Alberta, Edmonton (approximately 1.5 h). The next day, each rib eye was cut in half and each half was randomized to either 3 or 12 days of aging in a manner balanced for the anterior and posterior position. Aging was performed at 4^{o}C. At the end of each aging period, LT muscle halves were cut into 2.45 cm thick steaks (Figure 2.1) for pH, objective colour (CIE L*, a*, and b*), drip loss, cooking loss, cooking time, WBSF, and proximate analysis. The 1^{st} 2.45 cm steak from the posterior of the rib section was first used for recording objective colour measurement, cooking loss, cooking time and WBSF measurement. The 2^{nd} steak was removed and stored under vacuum in a polypropylene plastic bag at -20 °C for sensory analysis in another study. Three punctures were then made between the 3^{rd} and 4^{th} steaks for pH measurement (Figure 2.1) using an Accumet AP71 pH/Temperature Meter (Thermo Fisher Scientific, USA) fitted with a puncture electrode (Cat No. 655-500-30, FC210B,

Canada-wide Scientific, Ottawa, ON). The pH meter was calibrated before measurements using commercial standard buffer solutions of pH 4 and 7 (Thermo Fisher Scientific, USA). Three readings were taken for each sample and the average of these readings was used for statistical analysis. The 3rd steak was then removed and used for drip loss analysis and the 4th steak was frozen at -20 °C for proximate analysis, with the last steak packaged under vacuum and frozen at -20 C for intramuscular connective tissue (IMCT) analysis for a subsequent study. Following 12 days of aging, the remainder of the muscle was cut into 2.5 cm steaks from caudal to cranial ends. Again, the 1st steak was used for objective colour measurement, cooking loss, cooking time and for WBSF analysis, and the 2nd steak was vacuum packed and stored at -20 C for sensory analysis as part of another study. The 3rd steak was used for drip loss analysis, and three punctures were made between the 3rd and 4th steaks for pH measurement prior to removal of the 4th steak, which was frozen at -20 °C for proximate analysis. The 5th steak was used for IMCT analysis in another study.

2.2.3 Colour measurement:

The colour of the first steak was measured using a Minolta Chroma Meter (CR-400, Konica Minolta, Osaka, Japan). The instrument was calibrated before the first analysis of the day against a standard white calibration tile supplied by the manufacturer. The steak reserved for instrumental analysis of raw colour was allowed to oxygenate, or "bloom," at room temperature for approximately 20 min after removal from the muscle. After the bloom period, instrumental colour measurements of L*, a* and b* were obtained at 3 locations on the steak surface avoiding fat and connective tissue deposits. The 3 observations from each steak were averaged and the mean used for statistical analyses.

2.2.4 Cooking loss:

After colour measurement, the steaks were weighed to record the uncooked weight and then cooked on an electric clam-shell style grill (General Electric 4 in 1 Grill/Griddle, China) set at a temperature of 148.9^{o} C (300^{o} F). Steaks were cooked until the geometrical center of each steak reached 71°C. Steak temperature was monitored using a penetrating thermocouple (Tiny-Tag View 2s, Gemini Data Loggers, Chichester, West Sussex, UK) at the geometrical center of the steak. The time taken by the geometrical center of the samples to reach 71 °C was monitored using a stopwatch and recorded as the cooking time. Once 71 °C was reached, the cooked samples were immediately removed from the grill, placed in plastic bags and the bags immersed in ice water to halt the cooking process until the temperature at the geometrical center reached 20^{o} C. Once 20 °C was reached, the steaks were stored overnight in the plastic bags at 4°C. The following day, the weights of the steaks were recorded to determine the cooking loss. Cooking loss was determined where percent cooking loss = [(uncooked weight- cooked weight)/uncooked weight] × 100.

2.2.5 WBSF analysis:

After weighing the steaks for the estimation of cooking loss, the steaks were used for WBSF analysis. For WBSF analysis, eight 1.27 cm diameter cores approximately 2 cm long were removed parallel to the muscle fiber direction from each steak. Of these eight cores, six containing no large deposits of fat or connective tissue were selected and sheared once at their center, perpendicular to the fibers. The average of six readings was recorded as the WBSF value for each steak and was used in statistical analysis. Shear force measurement was performed at a blade speed of 200 mm/min using a Warner-Bratzler-like blade fitted to a Lloyd Instrument LRX plus (AMETEK, Digital Metrology Measurements, West Sussex, UK).

2.2.6 Drip Loss: Drip loss was measured using the technique described by Honikel and Hamm (1994). A steak was cut parallel to the muscle fiber direction of the *longissimus thoracis* muscle and associated adipose and epimysial tissue removed. The steak was weighed and suspended by means of a stainless-steel hook inside a plastic bag and the plastic bag was tied under atmospheric pressure. The samples were then held at 4ºC for 24 h. Bags were hung in such a way that the bag did not contact the meat. At the end of the measuring period, the muscle was taken from the pouch, dried gently with an absorbing tissue and reweighed. During weighing, care was taken that no condensation of water vapor occurred on the cold surface of the meat. Drip loss was expressed as the percentage of the initial weight of the meat.

2.2.7 Proximate analysis:

For proximate analysis, a 2.5 cm thick steak was trimmed of subcutaneous fat and epimysium and chopped into small cubes. The cubes were then placed in a pre-weighed aluminum tray, covered with an aluminum foil into which small slits were made with a knife. The trays were weighed, and the tray was then stored at −20 °C for freeze-drying. Following freeze-drying (VirTis freeze dryer, SP Industries/SP Scientific, Warminster, PA, USA), the aluminum trays containing the chopped meat cubes were weighed to calculate moisture lost during freeze-drying. The freeze-dried samples were then pulverized in a laboratory blender with three pellets of dry ice, packed in Whirl-Pak (Nasco Zefon International, Inc. Ocala, FL) bags and stored at −20 °C until further analysis. For total moisture, 2 ± 0.005 g of freeze-dried sample was heated in a glass vial at 100 °C in an oven for 18 h and then weighed to calculate total moisture in the raw meat. The crude fat content was measured as per Method 960.39, Association of Official Analytical Chemists (AOAC, 1995) in a Foss Soxtec™ 2050 (Foss Analytical, Hilleroed, Denmark). Approximately 2 (± 0.003) g were used to assess crude fat content, and the analysis was

performed in duplicate, with the average of the duplicates recorded as the fat content for statistical analysis. For crude protein content, 100 ± 5 mg of freeze-dried muscle in duplicate were analyzed for nitrogen content (Method 992.15; AOAC 1995) using a TruSpec Carbon/Nitrogen Determinator (LECO Corporation, St. Joseph, MI, USA). The crude fat and protein contents were then calculated as percentages of the raw meat and the mean of the duplicates used for statistical analyses.

2.2.8 Statistical analysis:

Meat quality characteristics were analyzed as a 4-factor randomized complete block, split-plot design. This factorial experiment has 16 treatment combinations of RFI (low/high) x HGP (yes, no) x RAC (yes/no) x day of aging (DOA)(Day 3, Day 12). Steers were the experimental unit for the main plot, while muscle half was the experimental unit for the subplots (two halves from each LT muscle) to which the DOA treatment (Day 3, Day 12) was applied. All data were analysed in R Studio (Version 1.1.456) software using a linear mixed effect model where the four treatments and their interactions served as fixed effects and animals within each harvesting date (kill) block served as the random effect. Harvesting date (kill) was included as a blocking factor as not all the steers were harvested on the same day. Initial weight before the first steroid implantation was used as a covariate to adjust for slight variation in the initial weight of animals. Correlation and variance structures were used where necessary to meet the assumptions of independence, normality, and homogeneity of residuals. The Shapiro-Wilks test was used to check the normality of residuals and residuals were plotted to check the assumption of homogeneity. Log transformations were used wherever necessary when the model assumptions were not met even after including correlation and variance structure. Data were reported as least

squares means. Tukey's Honest Significant Difference was used to compare least square means of different combination of treatments and significance was declared at $P \leq 0.05$ and tendencies from $0.05 < P < 0.1$. Pearson correlations between different meat quality characteristics were computed using the 'PerformanceAnalytics' package in RStudio to investigate linear relationships between various beef quality characteristics, and correlations were considered significant at $P < 0.05$.

2.3 Results:

One steer (low RFI, subjected to both ractopamine and steroid treatment) became lame and was removed from the study, resulting in the fourth kill having only 7 rather than 8 animals.

Intramuscular pH and temperature

Results for intramuscular pH and temperature at 1 and 3 h post mortem are presented in Tables 2.1, 2.2, and 2.3. Muscle temperature at 1 h post-mortem (T_{1h}) was not affected by RFI, HGP or RAC (Table 2.1, 2.2, 2.3, $P > 0.05$), and there were no significant interactions. The intramuscular temperature at 3 h post-mortem (T_{3h}), however, for the low RFI steers was higher than that of high RFI steers (Table 2.1, $P = 0.03$), but was not affected by HGP or RAC treatments (Tables 2.2 and 2.3, $P > 0.05$). Low RFI steers also had increased intramuscular pH at 1 h post-mortem (pH_{1h}) (Table 2.1, $P = 0.004$); however, this difference in pH did not persist at 3 h post mortem as the difference in mean pH values at 3 h post-mortem (pH_{3h}) for low and high RFI steer LT only approached significance (Table 2.1, $P = 0.07$). There were no effects of HGP and RAC on $pH_{1h.}$ and pH_{3h} (Tables 2.2 and 2.3, $P > 0.05$). The effect of RFI on intramuscular LT pH was completely diminished at measurement of the ultimate pH regardless of the post

mortem aging period, with no effect of RFI, HGP, RAC or aging on pH measured after aging (Tables 2.1, 2.2, 2.3 and 2.4).

Proximate analysis

The moisture content of LT muscle was unaffected by RFI and RAC (Table 2.1, 2.3, $P > 0.05$). There was an interaction between HGP and DOA for muscle moisture content, with the LT from steers that did not receive HGP having less moisture at day 12 post mortem than LT from steers that received HGP (Figure 2.2, $P = 0.01$). There were no effects of RFI or DOA on IMF (Table 2.1, 2.4, $P > 0.05$). The interaction between HGP and RAC for intramuscular fat content approached significance (Figure 2.3, $P = 0.06$). Within this interaction, the LT from steers that did not receive HGP but received RAC had less IMF than those that received no HGP and no RAC treatment, with LT from both of these groups not different from steers that were implanted regardless of RAC treatment.

For LT protein content, there was no effect of DOA (Table 2.4, $P > 0.1$), but there was an interaction between RFI x HGP x RAC (Table 2.5, $P < 0.002$). Within RAC fed steers, high RFI steers receiving steroids and low RFI steers not receiving steroids having higher mean protein contents than high RFI steers that did not receive steroids (Table 2.5).

Intramuscular colour

Mean L* value for LT from carcasses of low RFI steers were greater than that of high RFI steers (Table 2.1, $P = 0.007$), and mean L* values for HGP steers were less than that of steers that did

not receive HGP treatment (Table 2.2, P = 0.04). Mean L* values were not affected by RAC (Table 2.3, P > 0.05), but increased with DOA (Table 2.4, P < 0.001).

There was no effect of RFI, HGP and RAC on mean a* and b* values (Table 2.1, 2.2, 2.3, P > 0.1). The mean a* and b* values increased with post-mortem aging, however, indicating increased redness and yellowness at day 12 post-mortem (Table 2.4, P < 0.001).

Drip Loss

Low RFI steers had less drip loss than high RFI steers (Table 2.1, P = 0.009), but drip loss was not affected by HGP, RAC or DOA (Tables 2.2, 2.3, and 2.4) (P > 0.05).

Cooking Loss and Time

There was no effect of RFI, HGP or RAC on cooking loss or cooking time (Tables 2.1, 2.2, and 2.3) (P > 0.05). Cooking loss had a tendency to increase with DOA (Table 2.4, P = 0.056) and cooking time decreased with DOA (Table 2.4, P = 0.006) with the fastest cooking time observed on day 12 post-mortem.

Warner-Bratzler Shear Force: DOA was involved in two significant interactions for WBSF, interacting with HGP treatment (P = 0.03) (Figure 2.4) and RFI status (P = 0.04) (Figure 2.5). The LT from HGP steers had a greater mean WBSF value at day 3 post-mortem than non-implanted steers with the difference in WBSF declining with aging as there was no significant difference in mean WBSF values due to HGP by day 12 post-mortem (Figure 2.4). The interaction between DOA and RFI indicated that differences between LS means were because of

DOA as there was no difference in LS means between low and high RFI groups within day 3 or day 12 (Figure 2.5). RAC did not have any effect on WBSF (Table 2.3, $P > 0.05$).

Pearson correlations: Pearson correlations identifying relationships among different quality characteristics measured at day 3 post-mortem are presented in Table 2.6. At day 3 post-mortem, pH_{1h} was positively correlated to IMF ($r = 0.32$, $P < 0.05$) and to L* value ($r = 0.41$, $P < 0.01$) and negatively correlated to WBSF ($r = -0.34$, $P < 0.05$). T_{1h} was positively correlated to colour variable b* at day 3 post-mortem ($r = 0.39$, $P < 0.01$). Intramuscular ultimate pH was positively correlated with drip loss at day 3 post-mortem ($r = 0.30$, $P < 0.05$). Muscle moisture content was negatively correlated with IMF ($r = -0.51$, $P < 0.001$) and muscle protein at day 3 ($r = -0.79$, $P < 0.001$). Intramuscular fat content was negatively correlated to WBSF at day 3 ($r = -0.41$, $P < 0.01$). Muscle protein content was positively correlated to cooking time ($r = 0.30$, $P < 0.05$) and WBSF at day 3 ($r = 0.40$, $P < 0.01$). Colour variable L* showed a positive correlation with cooking loss at day 3 post-mortem ($r = 0.35$, $P < 0.05$). Colour variable a* showed a positive correlation with colour variable b* at day 3 ($r = 0.66$, $P < 0.001$). Cooking loss showed a positive correlation with cooking time ($r = 0.36$, $P < 0.05$) and WBSF ($r = 0.32$, $P < 0.05$) at day 3 post-mortem. Cooking time showed a positive correlation with WBSF at day 3 post-mortem ($r = 0.41$, $P < 0.01$).

Pearson correlations identifying relationships among different quality characteristics measured at day 12 post-mortem are presented in Table 2.7. At day 12 post-mortem, pH_{1h} was negatively correlated to the muscle moisture content ($r = -0.32$, $P < 0.05$) and positively correlated to intramuscular fat ($r = 0.33$, $P < 0.05$). T_{3h} was positively correlated to colour variable b* value at

day 12 post-mortem ($r = 0.44$, $P < 0.01$). Intramuscular pH measured at day 12 post-mortem was positively correlated to colour variable b* ($r = 0.29$, $P < 0.05$) and negatively correlated to cooking time ($r = -0.30$, $P < 0.05$) at day 12 post-mortem. Moisture content of LT was negatively correlated to IMF ($r = -0.75$, $P < 0.001$) and muscle protein ($r = -0.48$, $P < 0.001$) at day 12 post-mortem. Intramuscular fat was positively correlated to muscle protein at day 12 ($r = 0.42$, $P < 0.01$). Colour variable L* was positively correlated to colour variable b* ($r = 0.47$, $P < 0.001$) and cooking loss ($r = 0.34$, $P < 0.05$) at day 12 post-mortem. Colour variable a* was positively correlated to colour variable b* ($r = 0.46$, $P < 0.01$) at day 12 post-mortem. Colour variable b* was negatively correlated to WBSF ($r = -0.39$, $P < 0.05$) at day 12 post-mortem. Drip loss was positively correlated to cooking loss at day 12 post-mortem ($r = 0.31$, $P < 0.05$).

2.4 Discussion: In beef production, increased average daily gain, carcass weight, feed efficiency, and growth rate without additional fat deposition are important strategies that reduce overall production cost by producing more meat with less feed. Growth promotants like HGP and β-AA and selective breeding for RFI are efficient tools to increase carcass performance (Duckett and Pratt, 2014; Vestergaard et al., 1994; Schroeder, 2004; Herd et al., 2003; Johnston et al., 2002). These tools, however, may have adverse effects on meat quality (Roeber et al., 2000; Garmyn and Miller, 2014) and when used in combination may have deleterious implications (Ebarb et al., 2017). In the case of toughness induced by these growth promotants, corrective strategies like aging (Garmyn et al., 2014) and electrical simulation or tender-stretching to accelerate or augment the aging effect (Thompson, 2002) may have a beneficial effect in improving the palatability of the product.

Because cattle production strategies such as RFI, HGP and β-AA are important for ensuring the economical and efficient production of beef, it would be desirable for these factors to either improve or not affect beef eating quality. Interestingly, early post mortem muscle metabolism in the cattle selected for low RFI was affected, with the T_{3h} post mortem and the pH_{1h} post mortem increased relative to cattle selected for high RFI. These conditions were accompanied by increased L* values and reduced mean drip loss for the low RFI steer LT. The mechanism by which RFI selection may have increased meat L* value may have been due to an increased early post-mortem LT temperature (T_{3h}) in the carcasses of low RFI steers. High muscle temperature early post-mortem increases retention of oxygen by myoglobin in the post-rigor muscle as oxymyoglobin because high early post mortem temperature induces denaturation of electron transport chain proteins, reducing their ability to compete with myoglobin for oxygen (Sammel et al., 2002; Lawrie, 2006). Pearson correlations indicated a significant linear relationship between pH at 1 h post mortem and L* values at day 3 ($r = 0.41$), supporting that the increased pH at this time of the low RFI steer LT muscles contributed to the increased lightness of their LT muscles. The absence of significant correlations between T_{1h} and T_{3h} and L* indicated that temperature was most likely not determining L* value in the present study. Previous research investigating the effect of RFI selection on meat quality characteristics has not included measurement of early post-mortem muscle pH and temperature rate, so the effect of RFI on early post-mortem temperature and pH has not been examined by other authors.

In the current trial, low RFI steers had a lower mean drip loss than high RFI steers. Baker et al. (2006) and Arkfled et al. (2015) also reported reduced drip loss in the muscle of low RFI steers and low RFI pigs compared to that of high RFI steers and pigs respectively. The reduced drip

loss may have occurred through the LT of the carcasses of low RFI steers having higher pH_{1h} and a tendency for higher pH_{3h} values than that of the high RFI steers. As muscle pH increases away from the muscle protein isoelectric point, muscle proteins will be able to associate additional water to themselves, leading to reduced drip loss (Lawrie, 2003). However, the conclusion was not supported by Pearson's correlations as no negative correlation was found between pH_{1h}/pH_{3h}/ultimate pH and drip loss. The reason for Low RFI steers to have lower drip loss is unclear as there was a positive correlation between drip loss and ultimate pH at day 3 and there was no effect of RFI selection on ultimate pH at day 3 or day 12.

Results indicated that HGP treatment lowered the L* value of the LT, but it was only lowered by 0.73 units, an amount unlikely to be detected visually by consumers. The mean L* values of both groups (HGP/NO-HGP) was in the range of 35-38, however, which has been reported in the literature as producing a lightness of colour that is acceptable to consumers for beef (Lawrie, 1998). In contrast, selection for low RFI increased mean L* value which implied that RFI may be used to improve the lightness of LT. The results for the effect of RFI on L* values corroborate those reported by Lefaucheur et al. (2011) and Faure et al. (2013), who reported increased lightness in the muscle of low RFI pigs. The authors reported increased glycogen content in the *longissimus* muscle (LM) of RFI efficient pigs, which led to decreased ultimate pH and in turn lightened the colour of pork from the efficient pigs. In the present trial, glycogen content was not measured, and selecting for low RFI increased rather than decreased pH_{1h} and did not affect pH_{3h} or ultimate pH. Increased early post mortem intramuscular pH would be expected to decrease rather than increase L* value (Bruce and Ball, 1990). The mechanism by which RFI affected L* values is therefore unclear as there was a high positive correlation between pH_{1h} and L* values.

Although early post mortem muscle temperature did not affect LT L* values, the temperature of the LT at 1 h was positively correlated to LT b* value at days 3 and 12 post mortem, indicating that LT with increased temperature at this time would have an increased yellowness that persisted with days of aging. Low RFI steers had LT with a higher mean T_{3h} than that of high RFI steers, but this did not translate into a difference in mean b* values between low and high RFI LT. As mentioned previously, high temperature in early post mortem muscle may affect meat colour by increasing the rate of pH decline leading to low ultimate pH which improves meat colour by sparing oxygen through reduced mitochondrial respiration, allowing it to form oxymyoglobin (Young et al., 1999; Sammel et al., 2002). However, in this trial, there was no correlation between early post-mortem temperature (1 h and 3 h) and pH (1 h, 3 h, pH at day 3 and day 12) which supports there being no effect of RFI on b* value. Previous literature is inconsistent about the effect of RFI selection on muscle colour. Faure et al. (2013) reported greater L*, a* and b* values for pork from RFI efficient pigs compared to that of RFI inefficient pigs. On the contrary, Baker et al. (2006) reported greater b* values for beef strip loins from high RFI steers than from those of low or mid-RFI steers. Other literature suggests no direct effect of RFI on beef L*, a*, and b* values (McDonagh et al., 2001; Julimar do Sacramento Ribeiro et al., 2012; Zorzi et al., 2013; Reis et al., 2015; Fidelesetal., 2017).

That there was no effect of HGP on colour variables a* and b* was supported by Reiling et al. (2003), who reported no effect on muscle L*, a*, and b* values when steers were implanted once with Component TE-S, however, these authors reported diminished a* and b* values when cattle were implanted twice with TBA implants. No effect of β-AA on colour variables a* and b* were

supported by Bloomberg et al. (2013) who reported no effect of ZH on L*, a*, and b* values of *semimembranosus* but in *m. longissimus lumborum* (LL) there was a diminished a* value. Geesinketal, (1993) and Vestergaard et al. (1994) reported a trend towards pale meat by supplementing feed with β-AA in pigs and young bulls respectively.

With post-mortem aging, beef became redder (increased a* value), more yellow and less blue (increased b* value), and lighter (increased L* value). However, a* values of all the treatments at day 3 and day 12 post-mortem were in the normal range of 18-22 for beef (Lawrie, 1998).

As long as meat quality characteristics are within the expected normal ranges of appearance (colour) and functionality (pH), WBSF is one of the most important measures of eating quality acceptability as it estimates tenderness. Beef tenderness is the most important factor determining consumer acceptability of beef (Boleman et al., 1997; Kristina, 2000), and WBSF is usually correlated with trained taste panel assessments of beef tenderness (May et al., 1992; Shackelford et al., 1995). In this study, cooked LT from HGP steers had a greater mean WBSF value at day 3 post-mortem than non-implanted steers, with the difference in WBSF declining with aging until there was no significant difference in mean WBSF values due to HGP by day 12 post mortem. The decrease in toughness in beef with aging has been associated with proteolysis of muscle proteins by calpains, cathepsins, and caspases (Kemp et al., 2006; Houbak et al., 2008). Barham et al. (2003) reported that the consumer sensory tenderness of steaks from implanted steers did not differ from steaks from control steers after 7 days post-mortem which supports the absence of a difference in mean WBSF between HGP and NO-HGP steers at day 12 post-mortem in the present study. Ebarb et al. (2016) reported increased WBSF in implanted steers at day 3 post-

mortem but also noted that by day 14 post-mortem there was no difference in WBSF between the control group and implanted steers groups.

How much HGP increases beef toughness may depends upon whether the type of implant is estrogenic, androgenic or a combination of the two. This may be because of the different mechanism of increasing feed efficiency by different categories of implants, with the increase in the production and secretion of secondary hormones responsible for the accumulation of muscle proteins in cattle by implantation with estrogenic compounds and the reduced rate of muscle protein degradation by implantation with androgenic compounds. (Reinhardt, 2007). In this study, the increased toughness in steers implanted with HGPs was most likely because the second implant contained both estrogen and trenbolone acetate. Roeber et al. (2000) reported increased WBSF when cattle were subjected to a single implant similar to the second implant of this study. Foutz et al. (1989) reported that shear force values for implanted steers tended to be higher than for control steers, and steers implanted twice with trenbolone acetate had a greater likelihood of resulting in tough steaks than did steers implanted with a single trenbolone acetate implant or two estradiol implants. Gerken et al. (1995), however, reported that single estrogenic implants (20 mg of estradiol benzoate and 200 mg of progesterone) decreased the sensory tenderness of top sirloin steaks but a single androgenic combination implant (estradiol and trenbolone acetate) had no significant effect on beef sensory tenderness of top sirloin, but the authors reported no effect of any implant on WBSF. Similarly, Huck et al. (1991) reported no effect of estradiol and progesterone and/or TBA or doubly implant of TBA on WBSF. Results reported by Huck et al., (1991) and Gerken et al. (1995) might be affected by post-mortem aging as the authors measured WBSF after 6 and 7 days post-mortem respectively. Despite the results

of Gerken et al. (1995) and Huck et al. (1991), a recent meta-analysis of thirty-one experiments indicated that the use of HGP increased WBSF and that the increase in WBSF was cumulative with the number of times HGP implants were applied (Lean et al., 2018).

WBSF was positively correlated to cooking loss, negatively correlated to pH_{1h} and IMF at day 3 post-mortem but increased toughness of meat from implanted steers was not because of cooking loss, muscle pH or reduced IMF as there was no effect of steroids on these measures. HGP treatment did increase the protein content of the high RFI, RAC fed steers and WBSF was positively correlated to muscle protein at day 3 post-mortem, which suggests that induced toughness by HGP at day 3 post-mortem may be attributed to increased muscle protein in the LT of HGP steers. The increased toughness of beef imparted by HGP usually subsides to levels of toughness comparable to beef from cattle not treated with HGP by 14-21 days post-mortem through endogenous proteolysis (Barham et al., 2003; Ebarb et al., 2017), and the current study supports these previous results by not finding a correlation between muscle protein and WBSF at day 12 post-mortem. HGP treatment may have reduced calpain protease activity through increased calpastatin activity (Simmons et al., 1997), which may increases meat toughness (Strydom et al., 2009) as calpain activity has been positively associated with post-mortem tenderization of meat (Bhat et al., 2018). Growth promotants (GP) are also reported to increase hydroxyl pyridinoline content (Roy et al., 2015) which are a type of collagen crosslinks that are thermally stable and less soluble and contribute to increased cooked meat toughness (Weston et al., 2002). Another possible reason of increased toughness of HGP steers would be increased muscle fiber cross-sectional area by HGP treatment (Ebarb et al., 2016) which has been positively correlated with increased beef toughness (Crouse et al., 1991).

Selection for low RFI appeared to have little effect on the WBSF of the cooked LT. The interaction between RFI and post mortem aging affecting WBSF was predominated by the effects of aging rather than RFI, as there was no difference in WBSF due to RFI within the interaction. These results indicated no effect of RFI then on WBSF regardless of time aged post mortem. The results are supported by McDonagh et al. (2001), Baker et al. (2006), Reis et al. (2015), Ahola et al. (2011) and Fidelisa et al. (2017).

Supplementation with RAC did not show any significant effect on WBSF. Similar results were obtained by O'Neill (2001) who did not find any significant difference in shear force values in beef from control steers and steers fed ZH. Quinn et al. (2008) also found no difference in WBSF between steaks from control heifers or those that received RAC. Ebarb et al. (2017) reported that beef from steers that received both steroids and RAC was significantly tougher than beef from the control steers and from the HGP-treated steers. On the other hand, Boler et al. (2012) found that steaks from cattle fed RAC at four days post-mortem had 13% greater WBSF compared to controls, and Gruber et al. (2008) also reported increased WBSF for meat from steers fed RAC. Garmyn et al. (2014) observed that even though RAC increases WBSF in strip loins at day 14 post-mortem, RAC had a stronger response to aging with RAC steers producing beef that was more tender than that of control steers at day 21 post-mortem. Rathman et al. (2009) reported that the control group had lower WBSF values at all aging periods (7, 14, and 21 d) than cattle fed ZH ($P < 0.01$). Kellermeier et al. (2009) reasoned that when ZH was fed to cattle that received an estrogen and trenbolone acetate (TBA) terminal implant, ZH potentially increased LM WBSF because of induced muscle hypertrophy, the effects of which were reduced after an

aging period of 21 days. Bloomberg et al. (2013) also reported increased WBSF when cattle were fed with ZH. Leheska et al. (2009) reported that when ZH was supplemented in feed at 8.3 mg/kg (DM basis), there was an increase in left strip loin WBSF (3.3 vs. 4.0 kg). In all cases, the increased toughness by β-AA supplementation was under the acceptable limit of 4.5 kg, which is the transition point in consumer perception from tender to tough steak (Miller et al., 2001; Platter et al., 2003). Overall, the effect of β-AA on WBSF may be subject to the level used with no effect in this study at where steers received 200 mg/steer/day.

There was no effect of any treatment on cooking loss. Results are supported by other research work reporting no effect of steroids (Ebarb et al., 2017), β-AA, (Leheska et al., 2009; Garmyn et al., 2014) or RFI (Julimar do Sacramento Ribeiro et al., 2012) on the cooking loss when treatments were applied separately. In contrast, Baker et al. (2006) reported greater cooking loss percentages for steaks from low RFI steers than for those from mid-RFI steers.

Post-mortem aging affected cooking time, with cooking time decreased at day 12 post-mortem. Why this occurred was unclear, as cooking time at day 12 post mortem was negatively correlated with ultimate pH, and ultimate pH was not affected by post mortem aging. Cooking time at day 3 was positively correlated to protein content of the LT, suggesting that the determinants of cooking time differed between the two days. Post-mortem aging of meat involves proteolysis thus any effect of muscle protein on cooking time would be expected to be reduced with time post-mortem. This explanation is supported by no correlation being observed between muscle protein content and cooking time at day 12 post-mortem. No previous work has investigated the effect of post-mortem aging on cooking time.

Post-mortem rates of cooling and pH decline affect beef toughness, with high post-mortem muscle temperatures associated with increased rates of pH decline (Bruce and Ball, 1990) due to increased rates of glycolysis (Janz et al., 2001) and may lead to heat shortening if the pH falls below 6.0 while the muscle temperature is still above 35 ^{o}C (Thompson, 2002). In the current trial, low RFI steer LT were at a higher mean temperature at 3 h post mortem (T_{3h}) than high RFI steers, but the increased temperature did not fall into the heat shortening conditions window (pH < 6 when temperature > 35 ^{o}C) as the pH of all the steers was more than 6 when the temperature was more than 35 at 1-hour post-mortem. This is further substantiated by no correlation between early post-mortem temperature (1 and 3 h) and WBSF. In this trial, selection for RFI efficiency increased pH_{1h}, which was negatively correlated to WBSF at day 3 post-mortem (r = -0.34), suggesting a tendency for low RFI steers to have a lower WBSF than high RFI steers at day 3 post-mortem. Khan and Lent (1973) and Marsh et al. (1981) reported that, at an identical temperature at 2 h post-mortem, beef sensory tenderness increased by 2 units if pH_{3h} increased by 0.5 units. There was no difference between low and high RFI steers due to RFI in the present study, even in the interaction between RFI and DOA for WBSF. The results of the current study were similar to previous research that suggested no effect of RFI-efficiency on meat toughness (McDonagh et al., 2001; Baker et al., 2006; Reis et al., 2015 and Ahola et al., 2011). McDonagh et al. (2001) reported 13 % greater activity of calpastatin in low RFI steers, while no difference in calpastatin activity between efficient and inefficient steers was reported by Baker et al. (2006), suggesting no difference in WBSF between the two RFI types. Castro-Bulle et al. (2007) suggested that RFI efficient animals have low protein turnover and increased calpastatin activity in the muscle after 4-5 generations of selective breeding of low RFI animals (McDonagh et al.,

2001) which suggests that RFI could have an adverse effect on the shear force as toughness is related to the extent and rate of proteolysis. Within the population of cattle from which the steers from the current study were obtained, selection for RFI has been implemented for only one generation, hence, observation of no significant adverse effect of RFI on meat toughness is not unexpected. Previous research also suggested lower total collagen (Fideles et al., 2017; Zorzi et al., 2013) and greater soluble collagen in RFI-efficient cattle than RFI-inefficient cattle (Zorzi et al., 2013) which may increase toughness of low RFI steers as soluble collagen accounts for about 10% of the variation in tenderness (Culler et al., 1978), but this was not investigated in the present study.

The significant RFI x HGP x RAC interaction for LT protein content suggested that selection for RFI efficiency increased the protein content of steers that received RAC but were not implanted with HGP. This agrees with Castro-Bulle et al. (2007) who suggested lower protein turnover in low RFI animals than high RFI animals. There was no effect of RFI efficiency on IMF. This agrees with McDonagh et al. (2001), Baker et al. (2006) and Zorzi et al. (2013) who reported no effect of RFI on marbling scores and protein content. On the contrary, Ahola et al. (2011) reported greater marbling in inefficient steers than in RFI-efficient steers, although these authors mentioned that results could be contaminated by a sire effect. Growth promotants increase the lean muscle yields by acting as portioning agents, diverting nutrients towards lean (protein) muscle tissue rather than converting to adipose tissue, which leads to the expectation of higher protein content and lower fat deposition of cattle that receive GP (Ebarb et al., 2017; Duckett et al., 1999). In this study, HGP increased the protein content of high RFI, RAC fed steers but did not have any effect on intramuscular fat. Results are supported by Johnson et al. (1996) who used

an implant similar to the 2nd implant used in this study (120 mg TBA & 24 mg estradiol) and reported increased protein gain and no effect on the fat gain in implanted steers. Different implant strategies may have a different effect on marbling scores. Platter et al. (2003) reported increased marbling scores in steers which were not implanted before finishing phase or only received 2 implants than those with aggressive life time implant strategies or those receiving 4 or 5 implants respectively. However, all implant strategies in the study of Platter et al. (2003) had lower marbling scores than that of the control. Gergen et al. (1995) reported estrogenic implants may have lower marbling scores than androgenic and combination implants. Kellermeier et al. (2009) reported increased moisture and protein and decreased fat by both an estrogen + TBA terminal implant and β-AA or ZH. β-AA increase protein content by provoking muscle hypertrophy and through decreased muscle protein hydrolysis by increasing calpastatin activity (Wheeler and Koohmaraie, 1992). In this study, RAC had a tendency to reduce IMF content. The results are supported by Strydom et al. (2009) that reported both ZH and RAC may decrease LM fat with no change in the protein. Rathmann et al. (2009) found carcasses from cattle fed ZH had a lower percentage of fat and a greater percentage of protein and moisture. Garmyn et al. (2014) reported increased protein in ZH fed steers but no effect on RAC fed steers, however, Martin et al. (2012) found increased protein content and no effect on the fat content of both RAC or ZH fed steers. Leheska et al. (2009) suggested ZH operates as a repartitioning agent primarily through increased protein and muscle deposition. Overall, the literature suggests ZH is more likely to affect proximate composition than RAC.

2.5 Conclusion: Growth promotants are efficient ways to improve feed efficiency, however, they may have an adverse effect on meat quality. From the results of the current study, selection

of cattle for RFI efficiency may mitigate the adverse effect of HGP on the colour of meat. A cumulative effect on beef quality was not observed for selection for low RFI, HGP treatment and RAC supplementation, but the results should be viewed cautiously as the steers in the current study were selectively bred for low RFI for only one generation, and the interaction between HGP/RAC and RFI may change if the cattle are selectively bred for 4-5 generations. Aging appears to be an efficient method to mitigate toughness and diminished colour variables induced by growth promotants.

Figure 2.1 Position of different steaks used for meat quality characteristics analysis in the m. *longissimus thoracis*.

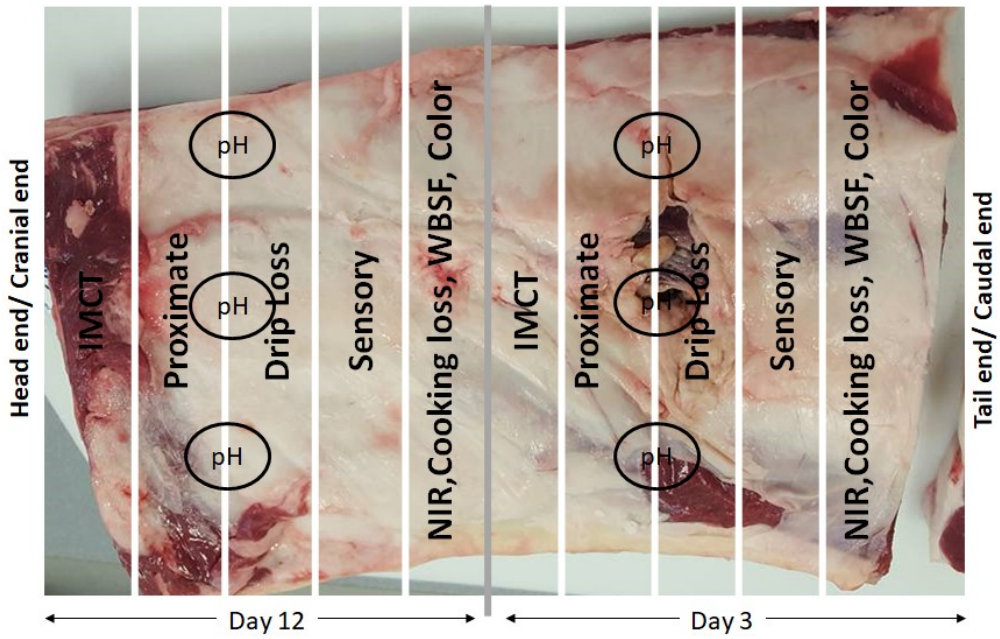

Figure 2.2 Effect of the interaction between steroid and post-mortem aging treatments on the mean moisture content (%) of the m. *longissimus thoracis* (P = 0.01).

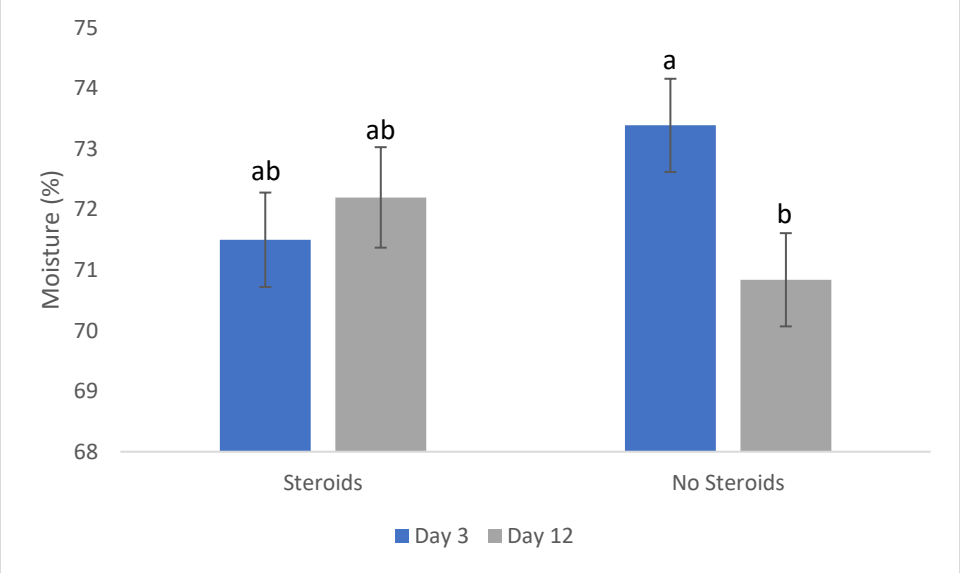

Figure 2.3 Effect of the interaction between steroid treatment and ractopamine treatment on intramuscular fat content in the m. *longissimus thoracis* (P = 0.05).

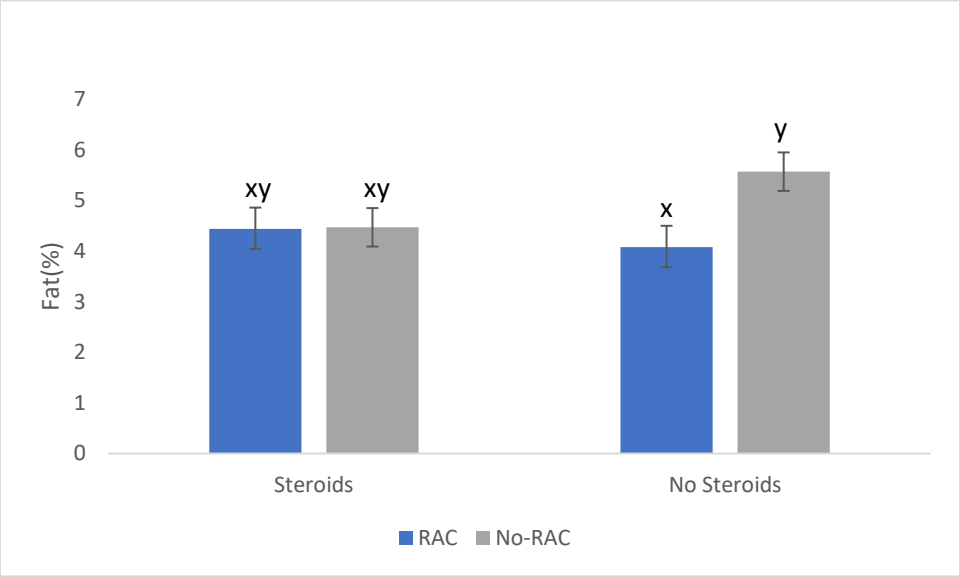

Figure 2.4 Effect of the interaction between steroid treatment and post-mortem aging on mean Warner Bratzler shear force (WBSF) values in the m. *longissimus thoracis* (P = 0.03).

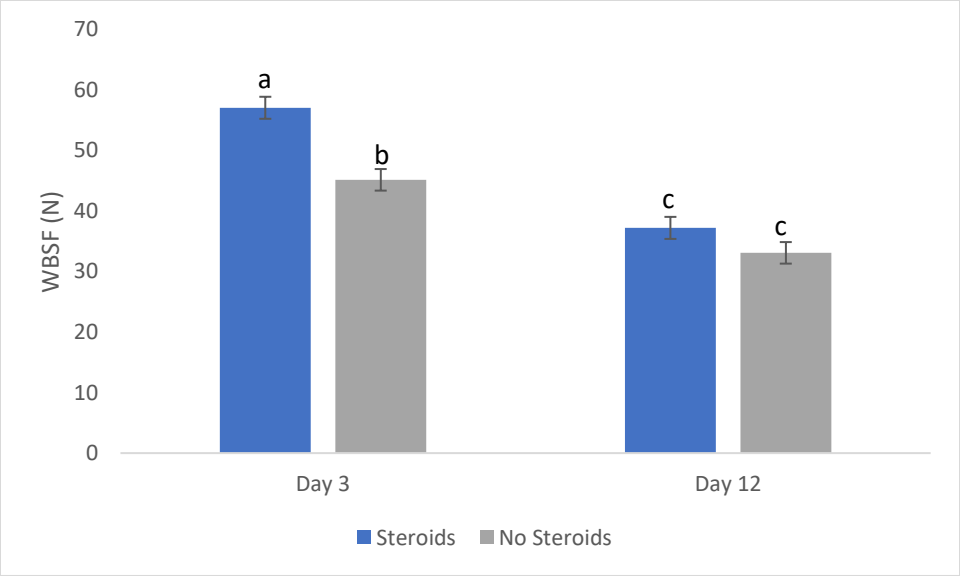

Figure 2.5 Effect of the interaction between selection for RFI and post-mortem aging on mean Warner Bratzler shear force (WBSF) values of the m. *longissimus thoracis* (P = 0.04).

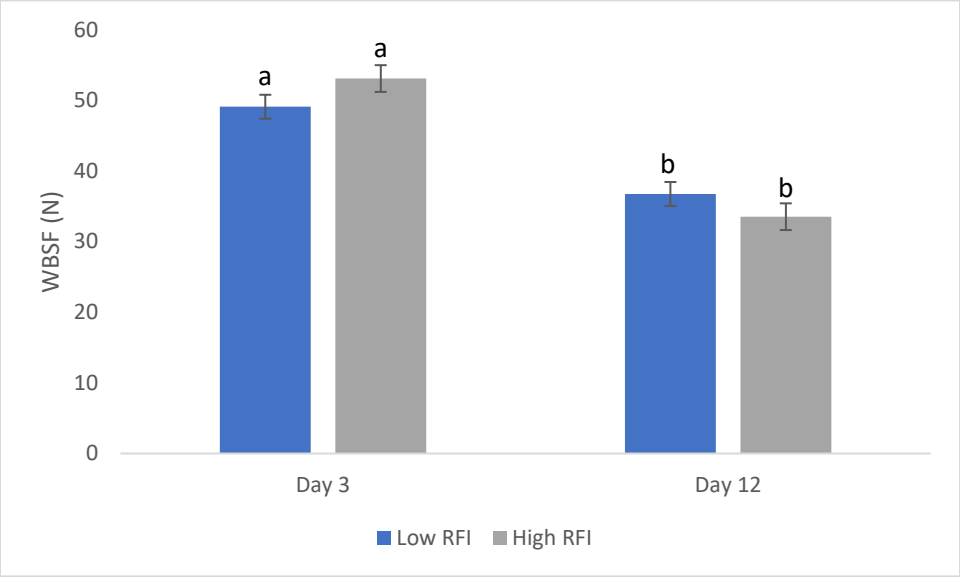

Table 2.1 Effects of RFI on beef quality characteristics of the m. *longissimus thoracis*.

Measurement	Low RFI	High RFI	P value
n	26	21	
T_{1h}	39.15 ± 1.09[1]	37.32 ± 1.20	0.20
T_{3h}	31.37 ± 0.49	29.68 ± 0.55	0.03
pH_{1h}	6.69 ± 0.04	6.55 ± 0.04	0.004
pH_{3h}	6.22 ± 0.04	6.11 ± 0.04	0.07
pH	5.59 ± 0.03	5.63 ± 0.03	0.21
Moisture (%)	71.16 ± 0.59	72.81 ± 0.67	0.054
Fat (%)	5.12 ± 0.31	4.46 ± 0.36	0.16
L*	35.93 ± 0.23	34.99 ± 0.25	0.007
a*	20.02 ± 0.21	20.13 ± 0.24	0.75
b*	3.82 ± 0.23	3.71 ± 0.26	0.72
Drip Loss (%)	1.30 ± 0.14	1.88 ± 0.16	0.009
Cooking loss (%)	17.22 ± 0.37	17.04 ± 0.08	0.71
Cooking Time (sec)	358.68 ± 11.86	350.95 ± 13.20	0.66

[1] Least square mean $\pm$ standard error of the mean.

Table 2.2 Effect of HGP on beef quality characteristics of the m. *longissimus thoracis*.

Measurement	Steroids	No-Steroids	P value
n	23	24	
T_{1h}	39.10 ± 1.15	37.37 ± 1.13	0.26
T_{3h}	31.14 ± 0.52	29.91 ± 0.51	0.08
pH_{1h}	6.62 ± 0.04	6.61 ± 0.04	0.71
pH_{3h}	6.17 ± 0.04	6.16 ± 0.04	0.94
pH	5.61 ± 0.03	5.61 ± 0.03	0.93
Fat (%)	5.12 ± 0.33	4.46 ± 0.35	0.16
L*	35.09 ± 0.24	35.82 ± 0.24	0.04
a*	19.89 ± 0.28	20.26 ± 0.22	0.23
b*	3.66 ± 0.24	3.87 ± 0.24	0.54
Drip Loss (%)	1.63 ± 0.15	1.55 ± 0.15	0.71
Cooking Loss (%)	17.29 ± 0.41	16.82 ± 0.39	0.32
Cooking Time (sec)	366.47 ± 12.70	343.16 ± 12.40	0.16

[1] Least square mean ± standard error of the mean.

Table 2.3 Effect of RAC on beef quality characteristics of the m. *longissimus thoracis*.

Measurement	RAC	No-RAC	P value
n	23	24	
T_{1h}	38.95 ± 1.15	37.52 ± 1.13	0.37
T_{3h}	29.96 ± 0.52	31.09 ± 0.51	0.16
pH_{1h}	6.63 ± 0.04	6.60 ± 0.04	0.57
pH_{3h}	6.13 ± 0.04	6.20 ± 0.04	0.24
pH	5.61 ± 0.03	5.61 ± 0.03	0.97
Moisture (%)	72.39 ± 0.64	71.57 ± 0.62	0.33
Fat (%)	4.56 ± 0.35	5.02 ± 0.34	0.36
L*	35.38 ± 0.24	35.53 ± 0.24	0.74
a*	20.18 ± 0.23	19.97 ± 0.22	0.56
b*	3.75 ± 0.24	3.78 ± 0.24	0.91
Drip loss (%)	1.60 ± 0.15	1.58 + 0.15	0.92
Cooking loss (%)	17.07 ± 0.41	17.04 ± 0.39	0.68
Cooking Time (sec)	356.76 ± 12.70	352.87 ± 12.40	0.83
WBSF (N)	43.25 ± 1.29	42.98 ± 1.26	0.74

[1] Least square mean ± standard error of the mean.

Table 2.4 Effect of post-mortem aging on beef quality characteristics of the m. *longissimus thoracis*.

Measurement	Day 3	Day 12	P value
n	47	47	
pH	5.61 ± 0.03	5.61 ± 0.03	0.80
Fat (%)	4.57 ± 0.35	5.01 ± 0.35	0.32
Protein (%)	20.92 ± 0.41	21.37 ± 0.42	0.43
L*	34.63 ± 0.24	36.29 ± 0.24	<0.001
a*	19.02 ± 0.22	21.13 ± 0.22	<0.001
b*	2.63 ± 0.24	4.90 ± 0.24	<0.001
Drip Loss (%)	1.71 ± 0.15	1.47 ± 0.15	0.30
Cooking loss (%)	16.45 ± 0.41	17.65 ± 0.39	0.056
Cooking Time (sec)	379.41 ± 12.55	330.22 ± 12.55	0.006

[1] Least square mean ± standard error of the mean.

Table 2.5 Effect of the HGP x RAC x RFI interaction on protein content of bovine m. *longissimus thoracis*.

Measurement		Low RFI		High RFI		P value
		Steroids	No Steroids	Steroids	No Steroids	
n	RAC	6	7	5	5	
Protein (%)	RAC	20.38 ± 0.80 [ab]	22.32 ± 0.73 [a]	22.99 ± 0.92 [a]	18.70 ± 0.87 [b]	0.002
n	No RAC	7	6	5	6	
	No RAC	21.72 ± 0.77 [ab]	20.51 ± 0.80 [ab]	21.26 ± 0.98 [ab]	21.24 ± 0.83 [ab]	

[1] Least square mean ± standard error of the mean.
[a,b] Means within a row with a different superscript are different (P < 0.05).

Table 2.6 Correlation coefficients between different beef quality characteristics at day 3 post-mortem.

	pH_{3h}	T_{1h}	T_{3h}	pH[8]	M[11]	IMF	P[10]	L*	a*	b*	DL	CL	CT	WBSF
pH_{1h}[1]	0.22	0.15	0.09	-0.02	-0.04	0.32*	-0.17	0.41**	-0.08	-0.01	0.08	-0.04	-0.15	-0.34*
pH_{3h}[2]		-0.18	0.07	0.05	0.01	0.08	-0.01	0.19	-0.02	0.09	-0.01	-0.01	-0.01	-0.18
T_{1h}[3]			0.40**	0.00	-0.12	0.14	0.00	-0.11	0.27	0.39**	0.07	-0.16	-0.01	-0.11
T_{3h}[4]				0.06	0.00	-0.21	-0.13	0.05	0.16	0.21	-0.06	0.00	0.26	0.20
pH[5]					0.08	-0.29	-0.29·	0.08	-0.00	-0.23	0.30*	0.00	-0.20	-0.08
M[11]						-0.51***	-0.79***	-0.23	0.03	-0.06	0.08	-0.26	-0.04	-0.06
IMF[6]							-0.10	0.22	0.24	0.16	-0.12	0.09	-0.26	-0.41**
P[7]								0.14	-0.21	-0.05	-0.12	0.27	0.30*	0.40**
L*									-0.13	0.23	-0.21	0.35*	0.28	-0.16
a*										0.66***	-0.03	0.02	0.03	-0.23
b*											-0.00	-0.12	0.19	-0.15
DL[8]												-0.26	-0.05	-0.19
CL[9]													0.36*	0.32*
CT[10]														0.41**

*Correlation differs from zero (P<0.05); ** Correlation differs from zero (P<0.01); ***Correlation differs from zero (P<0.001). [1]pH_{1h} = pH measured after 1 h post-mortem, [2]pH_{3h} = pH measured after 3 h post-mortem, [3]T_{1h} = Temperature measured after 1 h post-mortem, [4]T_{3h} = Temperature measured after 3 h post-mortem, [5]pH = pH measured on day 3 post-mortem, [6]IMF=Intramuscular fat, [7]P=Muscle protein, [8]DL = Drip loss, [9]CL = Cooking loss, [10]CT = Cooking time, [11]M=Moisture

Table 2.7 Correlation coefficients between different beef quality characteristics at day 12 post mortem.

	pH_{3h}	T_{1h}	T_{3h}	pH	M[11]	IMF	Protein	L*	a*	b*	DL	CL	CT	WBSF
pH_{1h}[1]	0.22	0.15	0.09	-0.25	-0.32*	0.33*	0.10	0.00	-0.21	-0.13	0.00	0.09	-0.03	-0.07
pH_{3h}[2]		-0.18	0.07	0.09	0.04	-0.16	-0.19	0.05	-0.19	-0.06	0.05	0.07	-0.08	0.17
T_{1h}[3]			0.40**	-0.18	-0.03	0.20	0.26	0.01	0.16	0.20	-0.04	-0.18	0.03	0.00
T_{3h}[4]				-0.11	0.01	0.03	0.00	0.06	0.15	0.44**	0.08	0.17	0.01	0.01
pH[8]					0.10	-0.04	-0.01	0.03	0.02	0.29*	0.24	0.07	-0.30*	-0.08
M[11]						-0.75***	-0.48***	-0.08	-0.03	-0.00	0.09	0.05	0.05	0.15
IMF[9]							0.42**	0.08	0.27	0.18	-0.20	-0.01	-0.04	-0.25
P[10]								-0.17	-0.05	-0.11	0.23	-0.03	-0.03	0.09
L*									-0.23	0.47**	0.02	0.34*.	0.03	-0.14
a*										0.46***	-0.24	-0.12	-0.04	-0.28
b*											0.06	0.24	-0.19	-0.39*
DL[7]												0.31*	-0.13	-0.01
CL[6]													-0.01	0.09
CT[5]														0.27

*Correlation differs from zero (P<0.05); ** Correlation differs from zero (P<0.01); ***Correlation differs from zero (P<0.001). [1]pH_{1h} = pH measured after 1 h post-mortem, [2]pH_{3h} = pH measured after 3 h post-mortem, [3]T_{1h} = Temperature measured after 1 h post-mortem, [4]T_{3h} = Temperature measured after 3 h post-mortem, [5]pH = pH measured on day 3 post-mortem, [6]IMF=Intramuscular fat, [7]P=Muscle protein, [8]DL = Drip loss, [9]CL = Cooking loss, [10]CT = Cooking time, [11]M=Moisture

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Chapter 3: Prediction of beef quality characteristics using polarized near infra-red spectroscopy

3.1 Introduction:

Eating quality of beef varies depending upon pre-slaughter and post-slaughter factors (Shorthose and Harris, 1990; Warriss, 1990; Bruce et al., 2004; Panea et al., 2018). Early post-mortem pH decline especially may dramatically affect meat quality and may lead to meat toughness defects like heat-shortening or cold-shortening if the rate of cooling of the carcass is too slow or too high respectively (Locker and Hagyard, 1963; Bruce and Ball, 1990; Dransfield, 1994; Ferguson et al., 2001). Meat toughness is the predominant quality factor in determining the overall acceptability of a meat product (Dikeman, 1987; Miller et al., 1995), and consumers are willing to pay a higher than usual price for beef that has tenderness guaranteed (Morgan et al., 1991; Boleman et al., 1997).

Meat toughness has been reported to be negatively affected by the selection of cattle for residual feed intake (RFI) or by the use of growth promotants (GP) in beef cattle such as hormonal growth promotants (HGP) (Simmons et al., 1997) and β adrenergic agonists (β-AA) (McDonagh et al., 2001; Castro Bulle et al., 2007). HGP and/or β-AA may inactivate proteases from the calpain family by excessive calpastatin activity (Simmons et al., 1997) which may increase meat toughness (Strydom et al., 2009) as calpain activity has been associated with the post-mortem tenderization of meat (Bhat et al., 2018). Ebarb et al. (2016) reported GP increased muscle fiber cross-sectional area, which has been associated with increased beef toughness as well (Crouse et al., 1991). GPs are also reported to increase hydroxyllysyl-pyridinoline content (Roy et al., 2015) which is a collagen crosslink that is thermally stable and associated with collagen that is less heat soluble and it may therefore contribute to increased cooked meat toughness (Weston et al.,

2002). Selection for low RFI cattle has also been reported to increase calpastatin activity and may lead to tougher meat after 4-5 generations of selective breeding of low RFI animals due to reduced calpain activity (McDonagh et al., 2001; Castro Bulle et al., 2007).

Other than meat toughness, consumers evaluate the eating quality of meat based on its colour, visible drip on the surface of the meat and the degree of marbling (Kropf et al., 1992; Becker, 2000). Traditionally, sensory analysis was used to evaluate eating quality of beef, but this requires a trained panel or hundreds of consumers and is often complex, expensive and requires the use and destruction of saleable product (Meilgaard et al., 2007; Vidal et al., 2014). Warner-Bratzler shear force (WBSF) analysis is a widely accepted objective method for tenderness measurement and it can show a high correlation with sensory panel scores (May et al., 1992) depending upon the muscle (Shackelford et al., 1995) but is time-consuming, requires trained personnel and destruction of samples (Guzek et al., 2013). Development of a reliable objective non-invasive method that could be used early post-mortem to predict the toughness of cooked beef would be an asset for beef processors. Hyperspectral imaging which integrates both spectroscopy and imaging techniques has been used to predict beef toughness with over 90% accuracy (Wu et al., 2012), but due to the problem of multicollinearity and computational challenges, it is difficult to implement this technique in on-line screening of meat (Elmasry et al., 2012). NIR spectroscopy has shown significant promise as a non-invasive, non-destructive technology to predict beef toughness (Mitsumoto et al., 1991; Park et al., 1998; Park et al., 2001; Rødbotten et al., 2001; Andrés et al., 2008; Ripoll et al., 2008) that requires fewer computing resources than digital imaging and is fast, sensitive, allows assessment of multiple meat quality parameters simultaneously and is suitable for on-line application (Osborne et al., 1993; Liu et al., 2003).

Although there have been several attempts to predict meat tenderness using NIR spectroscopy, most of them were not able to develop a reliable calibration algorithm for WBSF (Byrne et al., 1998; Leroy et al., 2004; Andrés et al., 2008; Prieto et al., 2009a). Luc et al. (2008) used polarized fluorescent light to detect the sarcomere length, as sarcomere length is correlated to meat toughness (Smulders et al., 1990) and found that polarized NIR light could be used to predict meat tenderness differences related to or induced by variation in sarcomere length. This author was not able to find any research since Luc et al. (2008) that used polarized NIR light to detect the non-sarcomere length induced meat toughness. This research will test the hypothesis that NIR light, when polarized, may predict the toughness and other meat quality measurements of beef with more than 80% accuracy. Specifically, the objective of this study was to test the accuracy of polarized NIR spectroscopy for the prediction of beef intra-muscular pH, colour, drip loss, cooking loss, intramuscular fat (IMF) of the raw product and Warner Bratzler shear force (WBSF) of the cooked product.

3.2 Material and methods

Experimental design and measurement of different meat quality characteristics are as described in the Material and Methods of Chapter 2 of this thesis (Section 2.2). To make this chapter self-sufficient, these are described again.

3.2.1 Animal Care: The animals used in this study were managed according to the University of Alberta's animal care and use program, which meets the national standards set by the Canadian Council of Animal Care (CCAC). The study was reviewed by a University of Alberta animal care and use committee prior to its execution (Animal Use Protocol number AUP00001801).

3.2.2 Experimental design: Forty-eight (48) Angus crossbred beef steers were randomly selected from the University of Alberta beef herd and were stratified based on body weight and

then randomized to treatment to minimize experimental error due to biological variation. Steers randomly available from the University of Alberta's two beef herds, 21 control (RFI = 0.59 ± 0.67) and 27 RFI efficient (RFI = 0.16 ± 0.58), were selected and stratified for weight. Cattle from each RFI group were randomly assigned to one of four treatments, specifically, steroid hormone growth promotants (HGP) only, β –adrenergic agonist (β-AA) only, both HGP and β-AA (COMBO), and controls which received neither HGP nor β-AA (CONT). The experiment was designed as a 2 (low, high RFI) x 2 (no, yes steroids) x 2 (no, yes β-AA) factorial. Steers were weaned at approximately 6 months old and were fed on a high forage diet for 120 days, after which they were stratified by weaning weight and randomly assigned to HGP and β-AA treatments. At a mean age of 320 days (10-11 months) and mean weight of 382.84 ± 19.74 kg, they were implanted with Component E-S (200 mg progesterone and 20 mg estradiol benzoate with 29 mg tylosin tartrate; Elanco Animal Health, Eli Lily Co., Greenfield, Indiana). Steers were then gradually alimented on to a high-grain finishing diet which consisted of 27% barley silage, 61% barley, 8% canola meal, and 4% mineral and rumensin pre-mix. Eighty (80) days after the first implant they were implanted with Component TE-S (120 mg trenbolone acetate (TBA) and 24 mg estradiol; Elanco Animal Health, Eli Lily Co., Greenfield, Indiana). Cattle received the same diet except for those from the β-AA and COMBO treatments, which were supplemented with RAC at manufacturer's recommended levels of 200mg/head/day for the 28 days before slaughter. The minimum difference between the second implant and initiating RAC supplementation was 65 days as Johnson (2014) suggested that implantation of beef cattle 60 to 90 days before feeding β-AA should have additive effects on lean tissue deposition.

Cattle were fed at the University of Alberta Roy Berg Kinsella Research Ranch, Kinsella, Alberta, until transported to and slaughtered at a provincially inspected abattoir in Alberta. Steers

were stratified by weight from heaviest to lightest prior to initiation of the RAC treatment into blocks with one animal per treatment in each block for slaughter. Cattle were slaughtered over 6 weeks with 8 cattle killed per week in a randomized complete block design. Following slaughter, carcasses were split into halves and the pH and temperature of the m. *longissimus thoracis* (LT, rib eye) were measured at 1 and 3 hours post-exsanguination by making three punctures on the right side of the carcass at the 12^{th} – 13^{th} rib with a puncture electrode (Cat No. 655-500-30, FC210B, Canada-wide Scientific, Ottawa, ON) and taking measurement at each puncture using an Accumet AP71 pH/Temperature Meter (Thermo Fisher Scientific, USA), with the averaged pH was used for statistical analysis. Following chilling of the carcasses for 48 hours, the carcasses were ribbed between the 12^{th}-13^{th} ribs and personnel trained in beef grading recorded from each carcass the 12^{th} and 13^{th} rib interface muscle score, grade marbling score, grade fat depth, and colour. Carcasses were then fabricated and the LT muscles were excised from the right side of the carcass. The LT muscles were packaged under vacuum in polypropylene bags and transported in ice-packed coolers to the University of Alberta, Edmonton (approximately 1.5 h). The next day, each rib eye was cut in half and each half was randomized to either 3 or 12 days of aging in a manner balanced for the anterior and posterior position. Aging was performed at 4^{o}C. At the end of each aging period, LT muscle halves were cut into 2.45 cm thick steaks (Figure 3.1) for pH, objective colour (CIE L*, a*, and b*), drip loss, cooking loss, cooking time, WBSF, and proximate analysis.

The 1^{st} 2.45 cm steak from the posterior of the rib section was first used for recording near-infrared spectra, for objective colour measurement, cooking loss, cooking time and WBSF measurement. The 2^{nd} steak was removed and stored under vacuum in a polypropylene plastic bag at -20 °C for sensory analysis (data used for a different study). Three punctures were then

made between the 3rd and 4th steaks for pH measurement (Figure 3.1), and internal muscle pH and the temperature were measured with an Accumet AP71 pH/Temperature Meter (Thermo Fisher Scientific, USA) fitted with a puncture electrode (Cat No. 655-500-30, FC210B, Canada-wide Scientific, Ottawa, ON)). The pH meter was calibrated before measurements using commercial standard buffer solutions of pH 4 and 7 (Thermo Fisher Scientific, USA). Three readings were taken for each sample and the average was used for statistical analysis.

The 3rd steak was then removed and used for drip loss analysis and the 4th steak was frozen at -20 °C for proximate analysis, with the last steak packaged under vacuum and frozen at -20 C for IMCT analysis for a subsequent study.

Following 12 days of aging, the remainder of the muscle was cut into 2.5 cm steaks from caudal to cranial ends. Again, the 1st steak used for collecting near-infrared spectra, for objective colour measurement, cooking loss, cooking time and for WBSF analysis, and the 2nd steak was vacuum packed and stored at -20 C for sensory analysis as part of another study, The 3rd steak was used for drip loss analysis, and three punctures were made between the 3rd and 4th steaks for pH measurement prior to removal of the 4th steak, which was frozen at -20 °C for proximate analysis. The 5th steak was used for IMCT analysis in another study.

3.2.3 Colour measurement:

After recording the NIR spectra, the colour of the steak used for NIR measurement was measured using a Minolta Chroma Meter (CR-400, Konica Minolta, Osaka, Japan). The instrument was calibrated against a standard white calibration tile supplied by the manufacturer before the first analysis of the day. The steak reserved for instrumental analysis of raw colour was allowed to oxygenate, or "bloom," at room temperature for approximately 20 min after removal from the muscle. After the bloom period, instrumental colour measurements of L*, a*

and b* were obtained at 3 locations on the steak surface avoiding fat and connective tissue deposits. The 3 observations from each steak were averaged and the mean used for statistical analyses.

3.2.4 Cooking loss:

After colour measurement, the steaks were weighed to record the uncooked weight and then cooked on an electric clam-shell style grill (General Electric 4 in 1 Grill/Griddle, China) set at a temperature of 148.9º C (300º F). Steaks were cooked until the geometrical center of each steak reached 71°C. Steak temperature was monitored using a penetrating thermocouple (Tiny-Tag View 2s, Gemini Data Loggers, Chichester, West Sussex, UK) at the geometrical center of the steak. The time taken by the geometrical center of the samples to reach 71 °C was monitored using a stopwatch and recorded as the cooking time. Once 71 °C was reached, the cooked samples were immediately removed from the grill, placed in plastic bags and the bags immersed in ice water to halt the cooking process until the temperature at the geometrical center reached 20º C. Once 20 °C was reached, the steaks were stored overnight in the plastic bags at 4°C. The following day, the weights of the steaks were recorded to determine the cooking loss. Cooking loss was determined where percent cooking loss = [(uncooked weight- cooked weight)/uncooked weight] × 100.

3.2.5 WBSF analysis:

After weighing the steaks for the estimation of cooking loss, the steaks were used for WBSF analysis. For WBSF analysis, eight 1.27 cm diameter cores 2 cm long were removed parallel to the muscle fiber direction from each steak. Of these eight cores, six containing no large deposits of fat or connective tissue were selected and sheared once at their center, perpendicular to the fibers. The average of six readings was recorded as the WBSF value for each steak and was used

in statistical analysis. Shear force measurement was performed at a blade speed of 200 mm/min using a Warner-Bratzler-like blade fitted to a Lloyd Instrument LRX plus (AMETEK, Digital Metrology Measurements, West Sussex, UK).

3.2.6 Drip Loss: Drip loss was measured using the technique described by Honikel and Hamm (1994). A steak was cut parallel to the muscle fiber direction of the *longissimus thoracis* muscle, and associated adipose and epimysial tissue were removed. The steak was weighed and suspended by means of a stainless-steel hook inside a plastic bag and the plastic bag was tied under atmospheric pressure. The samples were then held at 4ºC for 24 h. Bags were hung in such a way that the bag did not contact the meat. At the end of the measuring period, the muscle was taken from the pouch, dried gently with an absorbing tissue and reweighed. During weighing, care was taken that no condensation of water vapor occurred on the cold surface of the meat. Drip loss was expressed as the percentage of the initial weight of the meat.

3.2.7 Fat analysis:

For fat analysis, a 2.5 cm thick steak was trimmed of subcutaneous fat and epimysium and chopped into small cubes. The cubes were then placed in a pre-weighed aluminum tray, covered with an aluminum foil into which small slits were made with a knife. The trays were weighed, and the tray was then stored at −20 °C for freeze-drying. Following freeze-drying (VirTis freeze dryer, SP Industries/SP Scientific, Warminster, PA, USA), the aluminum trays containing the chopped meat cubes were weighed to calculate moisture lost during freeze-drying. The freeze-dried samples were then pulverized in a laboratory blender with three pellets of dry ice, packed in Whirl-Pak (Nasco Zefon International, Inc. Ocala, FL) bags and stored at −20 °C until further analysis. For total moisture, 2 ± 0.005 g of freeze-dried sample was heated in a glass vial at 100 °C in an oven for 18 h and then weighed to calculate total moisture in the raw meat.

The crude fat content was measured (Method 960.39, Association of Official Analytical Chemists (AOAC), 1995) in a Foss Soxtec™ 2050 (Foss Analytical, Hilleroed, Denmark). Approximately 2 (± 0.003) g were used to assess crude fat content, and the analysis was performed in duplicate, with the average of the duplicates recorded as the fat content for statistical analysis. The crude fat was then calculated as percentages of the raw meat.

3.2.8 NIR measurement: For spectra collection, an AQ6370C Optical Spectrum Analyser (Yokogawa, Tokyo, Japan) was used. Before recording the spectrum, the equipment was allowed to equilibrate for one hour, and then internal wavelength calibration was performed using the internal calibration light source. A 125 W, 120 V (Satco S4750) infrared heating lamp was used as a light source. An optical filter (FGL850S) was used to prevent the overtone effect during measurement. A fiber optic cable (Ocean Optics R600-7-VIS-125F) was used to transfer the reflected light from the sample to the spectrum analyser. A converter adapter (SMA905 to FC) was used to connect the optical fiber to the spectrum analyzer. The optical fiber was kept at a height of 10 cm above the measurement surface. A polarizer adapter was designed in the Electrical Engineering department workshop of the University of Alberta to hold a polarizer (LPIRE050-C linear polarizer, Thorlabs, Newton, New Jersey, USA, diameter 1.27 cm) which had a wavelength window from 1100 nm – 1650 nm and was assembled between the optical fiber probe and the measurement surface such that the reflected light from the measurement surface would pass through the polarizer to the optical fiber to be carried to the spectrum analyser. Spectra were collected in a dark room at 0.1 nm interval for 600 – 1700 nm. For each sample, spectra were collected in three modes – N, without an optical polarizer; P, with the polarizer axis parallel to the muscle fiber direction (at an angle of 180^{o}); and R, with the polarizer axis perpendicular to the muscle fiber direction (at an angle of 90^{o}). Six scans were

taken per sample. In each mode, two scans per sample were taken at two different locations on the steak. Infrared heating lamp was turned off for a minute between consecutive spectra collection to prevent heating of the sample. Prior to sample measurements, a Teflon sheet was used as a reference measurement surface for normalization as Teflon has a uniform distribution of spectral intensities. For both the Teflon references and meat samples, a black cover with a 4.5 cm diameter circular hole was used to illuminate only the circular area intended to be measured.

Spectra were expressed as reflectivity values by calculating the ratio of the reflected light intensity of the sample against that of the Teflon reference.

$R = I_{reflected}/I_{reference}$

Where R is the reflectivity of each wavelength, $I_{reflected}$ is the light intensity reflected from the meat sample and $I_{reference}$ is the light intensity reflected from the Teflon surface. Reflectivity values were subsequently used in statistical analysis as reflectivity adjusted for day to day machine variance and minor changes in the measurement surroundings. Teflon reference measurements were made without (N) and with (P) the polarizer and with the polarizer at a 90 ° rotation (R), with the appropriate reference used for measurements made under N, P or R conditions.

3.2.9 Statistical analysis and calibration development

Statistical results for the meat quality data were presented in Chapter 2. For NIR data, reflectivity values were used in all statistical analyses and as calculated were not expected to be of a value more than 1. Wavelengths with reflectivity values greater than 1 were assumed to be contaminated with fluorescence and were removed, leaving a wavelength range from 930 nm to 1650 nm. The spectra were recorded at a resolution of 0.1 nm, but only spectra recorded every 1

nm were retained for further statistical analyses. Smoothing using a coefficient of 11 was performed on the data before analysis to remove variation that could add unnecessary noise in the sample information.

Calibration R^2 and validation r^2 values describing linear relationships between NIR wavelengths and meat quality measurements were obtained using partial least square (PLS) analysis in Unscrambler™ (CAMO Software AS., Norway, Version 10.0.15063). Weighted regression coefficient plots were used to understand the degree of correlation between each wavelength and the response variable. Wavelengths with the higher regression coefficients were retained in the calibration prediction equations. PLS analysis used a segmented random cross-validation method to create calibration and validation equations. The entire sample population was available for construction of a calibration model in the PLS analysis and validation was performed within the PLS analysis by the software program randomly configuring 20 different sets samples with 2-3 samples in each set such that every sample was used once for a sub-data set to be used to validate the calibration model. Multiplicative scattering correction and 1st and 2nd order derivatives were performed on the data but were abandoned as they did not improve the r^2 value of models generated as compared to the model developed using un-processed data. PLS analysis was performed five times for each meat quality measurement and the NIR spectra within each mode (N, P, R) and the differences in R^2 and r^2 determined using one-way analysis of variance with mode as the sole source of variation with model significance at $P < 0.05$. Differences between the means of R^2 and r^2 for the N, P, and R modes were determined using Tukey's Honest Significant Differences test on the least square means, with significance at $P < 0.05$.

To determine differences between the means of reflectivity for the N, P and R modes, six specific wavelengths were selected from the entire wavelength range based on the observed peaks and trough in the collected spectra. Selected wavelengths included, 980 and 1200 nm which are observed as troughs and 1074 and 1274 nm which are observed as clear broad peaks, 1364 nm observed as a small peak in the collected spectra and 1450 nm was also included owing to the O-H first stretching overtone (Ortiz-Somovilla et al., 2006). Reflectivity data at six specific wavelengths - 980, 1074, 1200, 1274, 1364 and 1450 nm, for the N, P and R modes were analysed using a linear mixed model, with reflectivity as a response variable, mode as a fixed effect, harvesting date served as a random variable as not all spectra were collected on the same day. Differences between the means of reflectivity for the N, P and R modes at six specific wavelengths were determined using Tukey's Honest Significant Differences test on the least square means, with significance at $P < 0.05$.

The NIR of samples that represented the extremes of the various measurements were compared to identify areas within the spectra that may potentially be used to characterise sample differences. Five (5) samples with the highest and lowest L* were selected and their spectra collected at day 3 post-mortem in R mode were averaged, respectively, for comparison. Similarly, averaged spectra of 5 samples with the greatest a*, 5 samples with greatest b*, 5 samples with greatest WBSF, and 5 samples with greatest fat content were compared with 5 samples with the lowest a*, 5 samples with the lowest b*, 5 samples with the lowest WBSF, and 5 samples with the lowest fat content.

3.3 Results

Descriptive statistics for meat quality characteristics measured on LT at day 3 and 12 are shown in Tables 3.1 and 3.2, respectively. The HGP and RAC treatment given to the steers resulted in a wide range of variation in WBSF and other meat quality measurement values.

$I_{reference}$, reflected light intensity from the reference surface (Teflon disc) was recorded in N, P and R mode once per day of spectra collection to encounter the day to day instrument and environmental variation. The difference in reference Teflon spectra among N, P and R mode is shown in the Appendix (Section 5).

Results of PLS regression analyses performed on spectra obtained on day 3 post mortem regressed onto meat quality measurements performed on LT aged to day 3 post-mortem are shown in Table 3.3. For L* and a* values measured on day 3 post-mortem, spectra collected in P mode gave the highest prediction accuracy (validation $r^2 = 0.76$, 0.83 for L* and a*, respectively). For the L* value, there was no difference between the prediction accuracy in N and R mode, as both returned a validation r^2 of about 0.69 ± 0.10. For a*, N mode had the lowest prediction accuracy ($r^2 = 0.70$). For the colour variable b*, spectra collected in R and N modes gave the highest accuracy, with no statistical difference between the two modes ($r^2 = 0.83$), and P mode returned a validation r^2 of 0.81 ± 0.005 for b*. For drip loss measured at day 3, the largest r^2 was obtained in R mode, which characterized 89% of the samples with 74 % accuracy (calibration $R^2 = 0.89$, validation $r^2 = 0.74$). Spectra collected in N and P modes predicted drip loss with similar accuracy (calibration $R^2 = 0.86$ and 0.87; validation $r^2 = 0.70$ and 0.69 respectively). Again, for prediction of cooking loss measured at day 3, the largest r^2 was obtained in R mode, then in P mode and the lowest r^2 in N mode (calibration $R^2 = 0.94$, 0.92, and 0.76, validation $r^2 = 0.85$, 0.80, and 0.56 for R, P, N mode, respectively). For WBSF, NIR spectra

measured on day 3 samples without polarization (N mode) characterized 83% of the samples with 70 % accuracy (calibration $R^2 = 0.83$, validation $r^2 = 0.70$), while polarization with the polarizer axis parallel to the muscle fiber direction (P mode) characterized 81% of the samples with 59 % accuracy (calibration $R^2 = 0.81$, validation $r^2 = 0.59$). The largest r^2 was achieved on spectra collected with the polarizer axis perpendicular to the muscle fiber direction (R mode), which enabled characterization of 91% of the samples with 81% accuracy (calibration $R^2 = 0.91$, validation $r^2 = 0.81$). Intramuscular pH at day 3 was predicted with 79% accuracy in P and R mode and with 60% accuracy in N mode. Intramuscular fat at day 3 was best predicted in P and R mode compared to N mode ($r^2 = 0.81$, 0.83, and 0.77, for P, R and N mode respectively). Results of PLS regression analyses performed on spectra obtained on day 12 post-mortem regressed onto meat quality measurements performed on LT aged to day 12 post-mortem are shown in Table 3.4. For LT aged 12 days post-mortem, P mode predicted L* with a greater accuracy ($r^2 = 0.82$) than R mode ($r^2 = 0.76$), while rotation of the polarizer to 90° to the muscle fibre direction (R mode) showed greater accuracy for predicting a* ($r^2 = 0.72$) than P mode ($r^2 = 0.62$). Collection of spectra without polarization (N mode) produced the lowest validation r^2 of 0.67, 0.52 for L* and a* respectively. For b* measured at day 12 post-mortem, there was no difference between the r^2 values obtained in either N and P mode ($r^2 = 0.80$) but spectra collected in R mode gave a comparatively lower r^2 (0.78). For b*values, all three modes predicted its value with relatively similar accuracy, indicating that polarization had no effect on the characterization of the structures influencing the b* value of the beef. For drip loss at day 12, the greatest accuracy was achieved in R mode, then in N mode, with the least accuracy achieved in P mode ($r^2 = 0.73$, 0.66, 0.59, for R, N and P modes, respectively). For cooking loss at day 12, the greatest accuracy was achieved in R mode, then in N mode, with the least accuracy achieved in P

mode (r^2 = 0.73, 0.63, 0.61, for R, N and P modes, respectively). For WBSF at day 12, the greatest accuracy was achieved in R mode, then in N mode, with the least accuracy achieved in P mode (r^2 = 0.76, 0.72, 0.63, for R, N and P modes, respectively). For pH at day 12, the greatest accuracy was achieved in R mode, then in P mode, with the least accuracy achieved in N mode (r^2 = 0.84, 0.65, 0.50, for R, P and N modes, respectively). For IMF at day 12, the greatest accuracy was achieved in P mode, then in R mode, with the least accuracy achieved in N mode (r^2 = 0.78, 0.69, 0.56, for R, P and N modes, respectively).

Differences in the reflectivity values between the three modes at the six specific wavelengths are shown in Table 3.5. One-way analysis of variance indicated no difference in mean reflectivity at 980 and 1074 nm (Table 3.5). At 1200 and 1274 nm, mean reflectivity of R mode was greatest, followed by N mode, and the smallest reflectivity was that of P mode (Table 3.5). At 1358 nm and 1450 nm, mean reflectivity in R mode was greater than that in N and P modes (Table 3.5). Spectra of LT with the five highest and lowest L* values showed differences may have existed between 900 – 1350 nm and 1600 nm – 1650 nm (Figure 3.3). Differences in the reflectivity values between the three modes at the six specific wavelengths for the muscles with the five highest and five lowest L* values are shown in Table 3.6. One-way analysis of variance indicated no difference in mean reflectivity at 980 and 1074 nm (Table 3.6). At 1200 nm and 1274 nm, mean reflectivity of R mode was greater than that of P mode, with N mode not differing from either (Table 3.6). At 1364 nm and 1450 nm, mean reflectivity in R mode was greater than that in N and P mode (Table 3.6).

Spectra of LT with the five highest and lowest a* values showed differences may have existed between 900-930 nm, where the averaged reflectivity of samples with the lowest a* was greater than that of the highest a*. Conversely, from 960 – 1130 nm, the averaged spectrum of the

samples with the highest a* was greater than that of the lowest a*. The reflectivity of the averaged spectrum of the five samples with the lowest a* rose again at around 1330 nm and remained greater than the averaged spectrum of the five LT samples with the highest a* reflectivity (Figure 3.4). Differences in the reflectivity values between the three modes at the six specific wavelengths for the muscles with the five highest and five lowest a* values are shown in Table 3.7. One-way analysis of variance indicated no difference in mean reflectivity at 980, 1074 and 1200 nm (Table 3.7). At 1274 and 1450 nm, mean reflectivity in R mode was greater than that in P and N mode (Table 3.7). At 1364 nm, mean reflectivity of R mode was greater than that of N mode, with P mode not differing from either (Table 3.7).

Spectra of LT with the five highest and lowest b* values showed that the reflectivity of the five highest b* was higher than that of the five lowest b* at all wavelengths, which suggests differences may exist between 900 – 1650 (Figure 3.5). Differences in the reflectivity values between the three modes at the six specific wavelengths for the muscles with the five highest and five lowest b* values are shown in Table 3.8. One-way analysis of variance indicated no difference in mean reflectivity at 980 and 1074 nm (Table 3.8). At 1200, 1364 and 1450 nm, mean reflectivity in R mode was greater than that in P mode with N mode not differing from either (Table 3.8). At 1274 nm, mean reflectivity of N and R mode was similar and greater than that of P mode (Table 3.8).

Spectra of LT with the averaged five greatest and averaged five smallest WBSF values showed differences may have existed between 900 and 1000 nm and from about 1350 to 1650 nm (Figure 3.6). Differences in the reflectivity values between the three modes at the six specific wavelengths for the muscles with the five greatest and smallest WBSF values are shown in Table 3.9 One-way analysis of variance indicated no difference in mean reflectivity at 980 and 1074

nm (Table 3.9). At 1200 nm and 1274 nm, mean reflectivity of R mode was greater than that of N and P mode (Table 3.9). At 1364 nm, mean reflectivity in R mode was greater than that in N mode, with P mode not differing from either (Table 3.9). At 1450, mean reflectivity of R mode was greater than that of N mode but was similar to that of P mode (Table 3.9).

Figure 3.7 shows that reflectivity of the five samples with the lowest intramuscular fat content was greater than that of the five LT samples with the highest intramuscular fat content from about 900 -1000 nm, about 1200 nm and then from about 1340 – 1650 nm. Differences in the reflectivity values between the three modes at the six specific wavelengths for the muscles with the five highest and five lowest fat content are shown in Table 3.10. One-way analysis of variance indicated no difference in mean reflectivity at 980 and 1074 nm (Table 3.10). At 1200 and 1364 nm, mean reflectivity in R mode was greater than that in N mode, with P mode not differing from either (Table 3.10). At 1274 and 1450 nm, mean reflectivity of R mode was greater than that of N and P mode (Table 3.10).

3.4 Discussion

Typical absorbance spectra show broad peaks at specific wavelength bands corresponding to the vibration of O-H, C-H and N-H bonds. In reflectance spectra, these broad absorbance bands appear as troughs or minima. The reflectance band for the O-H bond was observable as a trough at around 980 and 1450 nm owing to the second and first stretching overtones of the O-H bond respectively (Ortiz-Somovilla et al., 2006; Morsy and Sun, 2013). The absorption of the C-H bond was observed as a minimum at 1200 nm because of the 2nd overtone of the C-H bond (Park et al., 2001; Morsy and Sun, 2013). These characteristic bands for water and the C-H bond are similar to those reported by Andres et al. (2008), Leroy et al. (2003) and by Cozzolino and

Murray (2004). However, the authors received peaks rather than troughs for the characteristic bands of water and C-H absorption as they reported absorbance spectra (log(1/R)) rather than reflectivity.

N, P and R modes showed that reflectivity was greater in R mode than in P and N modes at 1200, 1274, 1364 and 1450 nm, confirming that the direction of spectral reflectance can affect NIR reflectivity spectra. The angle of polarization relative to the muscle fiber direction appeared to influence the accuracy of the calibration models. Spectra collected in R mode at day 3 post-mortem gave the largest validation r^2 for WBSF, drip loss and cooking loss, while colour measurements L* and a* were best predicted in P mode. IMF and pH were also best predicted using polarization, with no difference between P and R mode indicating that muscle fibre direction did not influence the measurement. In the case of WBSF, the calibration models developed with parallel polarization described less variation than calibration models without polarization, whereas with intramuscular pH at day 3 and day 12, using parallel polarization gave models with greater validation r^2 than without polarization. These results indicated that muscle fibres and their direction influenced the prediction of WBSF and pH. The presence of myofibrils may affect the reflection and scattering of light because myofibrils themselves may act as optical fibres and can conduct the light along their length by a series of internal reflections due to the difference in refractive indices of myofibrillar (high refractive index) and sarcoplasmic fluid (low refractive index) (Prieto et al., 2009; Swatland, 2012). Polarization of the light reflected from the meat sample/reference material (Teflon) reduces the glare and improves the quality of the signal entering the spectrophotometer and generates spectra free from a multi-scattering effect in the region of polarization (Gobrecht et al., 2016).

An unexpected result of this research was that NIR spectroscopy could, with some accuracy, predict beef colour. This may have occurred because colour variables are correlated to intramuscular fat content and muscle pH (Prieto et al., 2009a). Results of the current study are supported by Prieto et al. (2008), as those authors used wavelengths from 1230 –1400 and 1600 – 1710 nm (corresponding to C-H overtones and combination bonds) to predict colour variables L* and b* of beef from young cattle with 86 % and 90 % accuracy respectively. Leroy et al. (2003) also predicted colour variables L* and b* with 85% and 75% accuracy, respectively, using a wavelength range of 833–2500 nm. Near-infrared wavelengths are longer than those visible to the human eye, which range from 400 to 700 nm. Andrés et al. (2008) predicted L* with 85% accuracy using both visible and NIR regions while Cozzolino et al. (2003) and Liu et al. (2003) used both NIR and visible wavelength regions (400-700 nm) to successfully predict the a* value of beef, achieving an r^2 of 0.93 and 0.90 for pork and beef respectively. The use of the visible light wavelength region may have enhanced prediction of the meat a* values as a* is related to myoglobin concentration (Prieto et al., 2009a) and myoglobin is usually predicted using wavelengths from the visible region because the different colours of the derivatives of myoglobin (bright red, purple and brown for oxymyoglobin, deoxy myoglobin, and metmyoglobin respectively) are easily detected in that range (Ripoll et al., 2008).

Variation in reference data can increase accuracy of the NIR prediction models. Post-mortem aging improved meat colour as L*, a* and b* values were greater at day 12 post-mortem than that at day 3 post-mortem. As a result, the standard deviation of the reference L* value increased with aging, that of a* decreased with aging and that of b* was similar to that of day 3 post-mortem. This change in the standard deviation of the reference data can be seen in the NIR calibration, which shows that the predictability of NIR calibration model increased for L*,

decreased for a* and was similar for b* at day 12 post-mortem when compared to the r^2 value of prediction models at day 3 post-mortem. The results are supported by Prieto et al. (2008) who reported high correlation coefficient between absorbance data and colour variables L*, a* and b* in beef from young cattle but not in adult steers because of the wider range of variability of colour variables reference data in the young cattle data than in that from the adult steers.

Drip loss and cooking loss were predicted with an accuracy of up to 74% and 85 % at day 3 post-mortem. Because NIR spectroscopy cannot directly predict water holding capacity, a moderate coefficient of determination for cooking loss may be attributed to the relationship between water holding capacity and the chemical composition of meat as water holding capacity correlates with absorbance data at wavelengths related to water, fat and muscle protein (Prieto et al., 2008). Previous research reported a limited ability of NIR spectroscopy to predict drip loss and cooking loss (De Marchi et al., 2007; Geesink et al., 2003; Hoving-Bolink et al., 2005; Savenije et al., 2006; Leroy et al., 2004; Andrés et al., 2008), although Forest et al. (2000) predicted drip loss with an accuracy of 84% using NIRS in the range of 900-1800 nm in porcine *longissimus dorsi* muscles with fiber optic sampling. Specific pre-treatment of spectra to reduce day to day changes in instrument response, increasing the number of scans to 30 per spectrum and high variation of 0.9-16% in the reference drip loss data may have helped Forest et al. (2000) to predict drip loss with high accuracy.

Most of the previous work using NIR spectroscopy to characterize or predict the WBSF of the cooked product has been unsuccessful (Park et al., 1998; Venel et al., 2001; Leroy et al., 2004; Andrés et al., 2008; Ripoll et al., 2008; Prieto et al., 2008). Limited variation in the WBSF reference data, poor precision of the reference method, and the use of homogenized or minced meat rather than intact steaks may have contributed to previous poor coefficient of correlations

between NIR absorbance data and WBSF (Prieto et al., 2009a). In this study, intact beef steaks were used for NIR spectroscopy and from these WBSF could be predicted with more than 80% accuracy at day 3 post-mortem in R mode. Collecting spectra from intact meat slice has an advantage over the use of minced meat for the prediction of WBSF as meat toughness is associated with the muscle fiber cross-sectional area and the composition of muscle fiber type (Crouse et al., 1991), and mincing breaks down the structure of the muscle and fibre direction, destroying the very structure determining the force required to shear it. The broad range of WBSF reference data provoked by growth promotants and the advantage of using the same steak for recording the spectra and for the reference WBSF measurement might have contributed to the moderate prediction of WBSF. In this study, WBSF was negatively correlated to intramuscular fat and positively correlated with muscle protein, supporting correlations by others between WBSF and absorbance at NIR wavelengths associated with C-H bond. Prieto et al. (2008) observed the highest correlation coefficient between WBSF and absorbance data at 1300-1400 nm, which is the region of wavelengths associated with the C-H bond. Aging produces noticeable improvements in tenderness (Leroy et al., 2004) and as expected in the current study, day 12 samples had lower mean WBSF values than at day 3 post-mortem. There would be an expectation that these structural changes would be detected using NIR and that the prediction of WBSF by NIR would be the same regardless of whether measured on day 3 or day 12 post mortem. There was a numerical decrease however in the validation coefficient of determination for WBSF from day 3 to day 12, suggesting that whatever features of the meat structure NIR was able to detect actually contributed less to prediction of WBSF. No correlation between WBSF and IMF/muscle protein was observed at day 12 post-mortem supports this conclusion and the reduced coefficient of determination for WBSF at day 12 observed.

Although prediction accuracy of WBSF of 81% was achieved in day 3 post mortem product, other measurements such as pH may allow for indirect prediction of WBSF or sensory tenderness. Intramuscular pH has been related to tenderness of beef (Bruce and Ball, 1990) and may be useful for sorting carcasses anticipated to produce tough beef. Intramuscular pH is also related to final product colour (Young et al., 1999; Sammel et al., 2002) and may be useful then for predicting final product colour as well. Swatland et al. (1995) reported that light scattering properties of muscle tissue are affected by tissue pH, which explains the 79 % accuracy in prediction of pH at day 3 in P and R mode and the 84 % accuracy of prediction of pH at day 12 in R mode . As reviewed by Prieto et al. (2009), most researchers may not able to successfully predict pH using NIR spectroscopy as they collected spectra from a ground sample or due to a narrow range of pH values in the reference data. Andres et al. (2008) successfully predicted pH at 24 h post-mortem with an r^2 of 0.97, the heightened accuracy of which may be attributed to a wide range of reference pH values (5.50-6.67) and to the meat samples being measured while intact rather than in a ground form.

NIR spectroscopy has been commercialized as a method of predicting the fat content of homogenized meat. Previous research has confirmed the ability of NIR spectroscopy to successfully predict intramuscular fat (Cozzolino et al., 2002; Cozzolino and Murray, 2002) and De Marchi et al. (2007) predicted intramuscular fat of minced beef with an accuracy of 99%. In this study, the limited accuracy of 83% and 78% at days 3 and 12 respectively observed in the present study could be attributed to the heterogenous nature of beef. Collecting spectra in minced meat has been shown to increase the accuracy of prediction as mincing may improve the homogeneity of the samples (Cozzolino and Murray, 2002; Prevolnik et al., 2005; Barlocco et

al., 2006). Increasing the number of scans in intact beef samples may also help to compensate or account for the uneven distribution of intramuscular fat in muscles (Ripoll et al., 2008).

3.5 Conclusion: The results of the present study indicate that polarization of NIR light significantly increases the accuracy of NIRS. WBSF was best predicted with more than 80% accuracy when the polarizer axis was perpendicular to the muscle fiber direction. In this study, the range of reference WBSF data was extended by introducing variation in meat toughness by the use of growth promotants. However, low precision of the reference method may contribute to reduced accuracy of calibration models. Slice shear force measurement may be more repeatable and more correlated with tenderness obtained by sensory analysis than the WBSF method (Shackelford et al., 1990). In future research, slice shear force may be used in place of WBSF as a reference method to measure meat toughness to reduce error in calibration development due to low precision of the reference method. This study supports the use of polarization in NIR spectroscopy to improve the prediction of beef quality characteristics, particularly L*, a*, b*, cooking loss, WBSF, pH and IMF.

Figures:

Figure 3.1. Position of different steaks used for meat quality characteristics analysis.

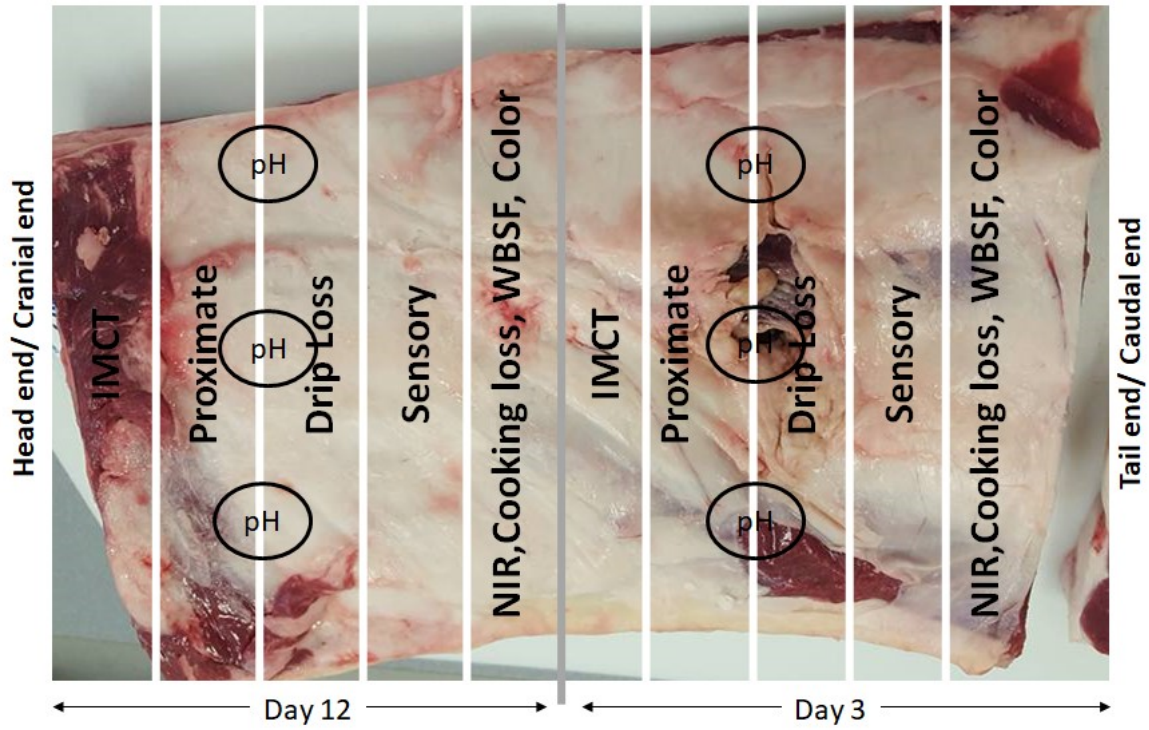


Figure 3.2.Averaged spectrum of 47 muscles collected at day 3 post mortem in N, P and R mode. N, spectra collected with no polarizer; P, spectra collected with polarizer axis parallel to the mus cle fiber direction; R, spectra collected with polarizer axis at 90 °angle to muscle fiber direction.

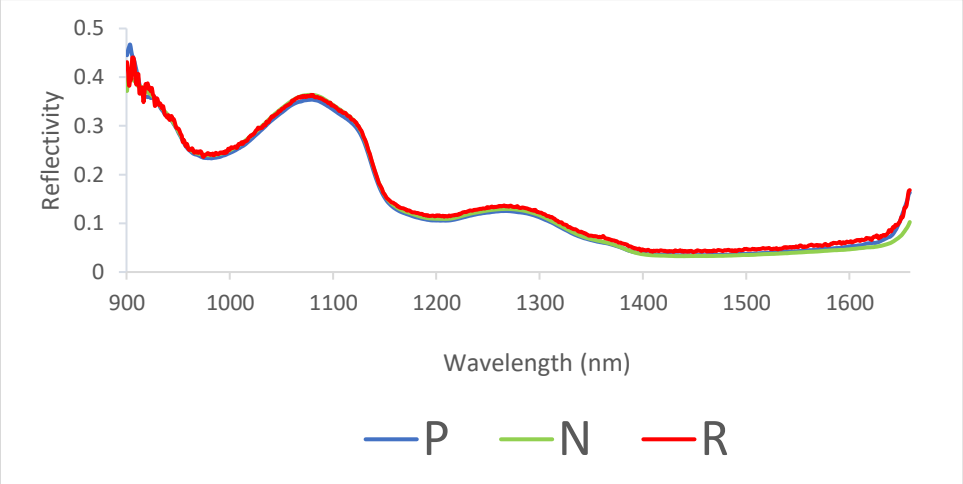


Figure 3.3: Averaged spectrum of 5 samples with highest L* and 5 samples with lowest L* at da y 3 post-mortem in R mode. R, spectra collected with polarizer axis at 90 °angle to muscle fiber direction.

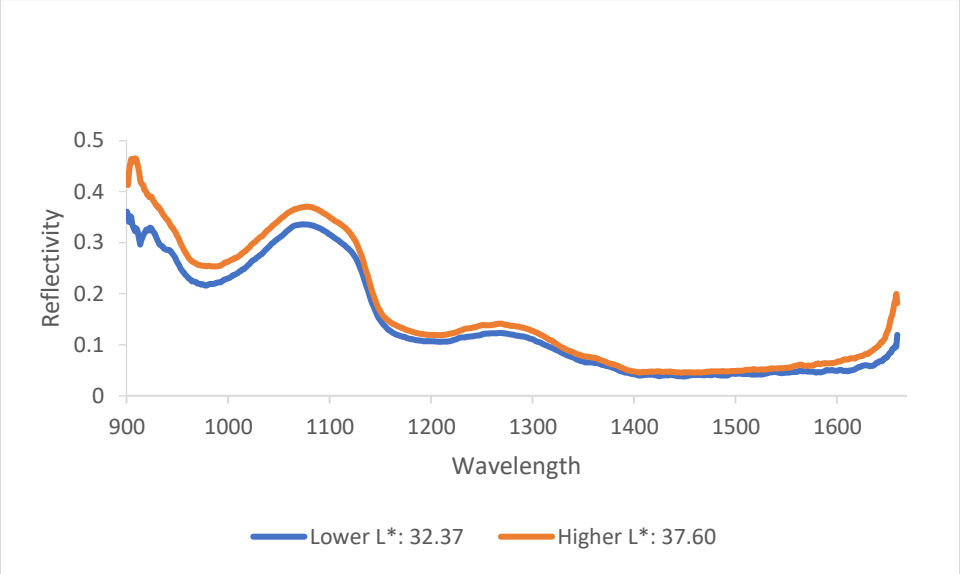


Figure 3.4: Averaged spectrum of 5 samples with highest a* and 5 samples with lowest a* at day 3 post-mortem in R mode. R, spectra collected with polarizer axis at 90 °angle to muscle fiber dir ection.

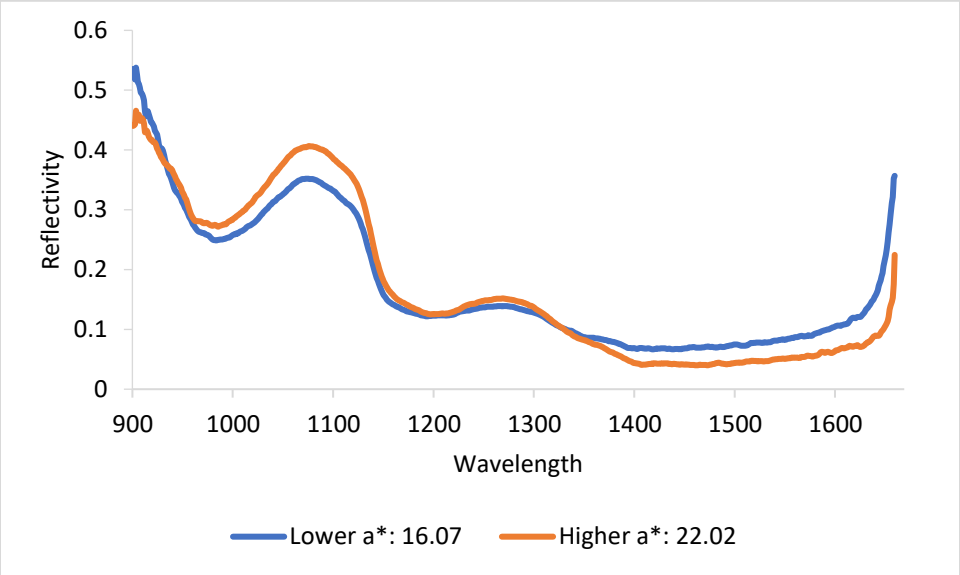


Figure 3.5: Averaged spectrum of 5 samples with highest b* and 5 samples with lowest b* at day 3 post-mortem in R mode. R, spectra collected with polarizer axis at 90 °angle to muscle fiber dir ection.

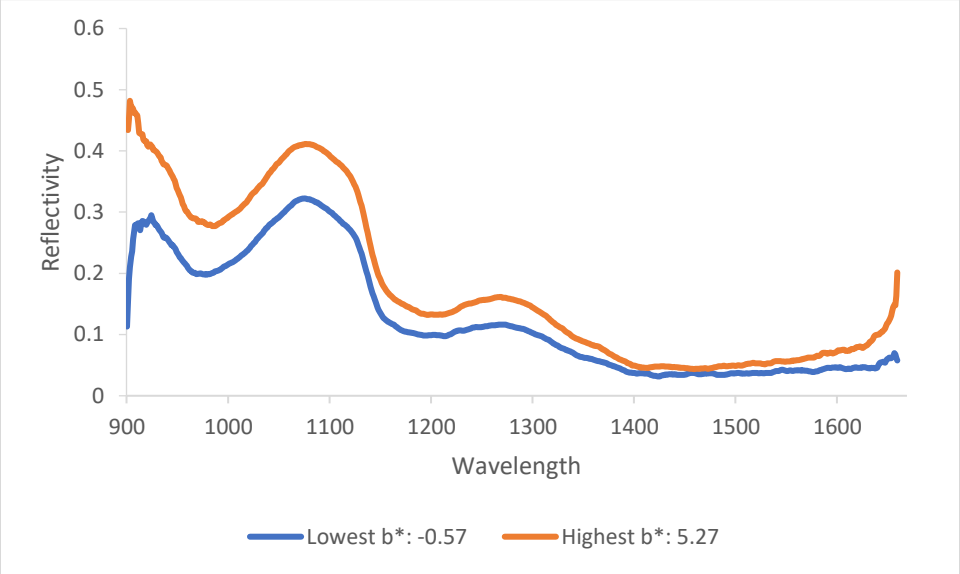


Figure 3.6: Averaged spectrum of 5 most tender and 5 toughest samples at day 3 post-mortem in R mode. R, spectra collected with polarizer axis at 90 °angle to muscle fiber direction.

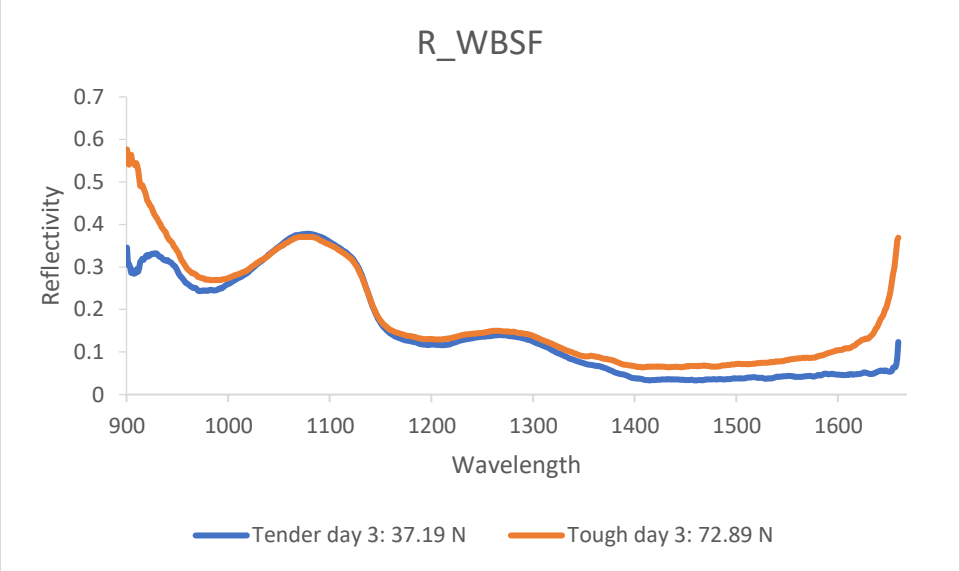
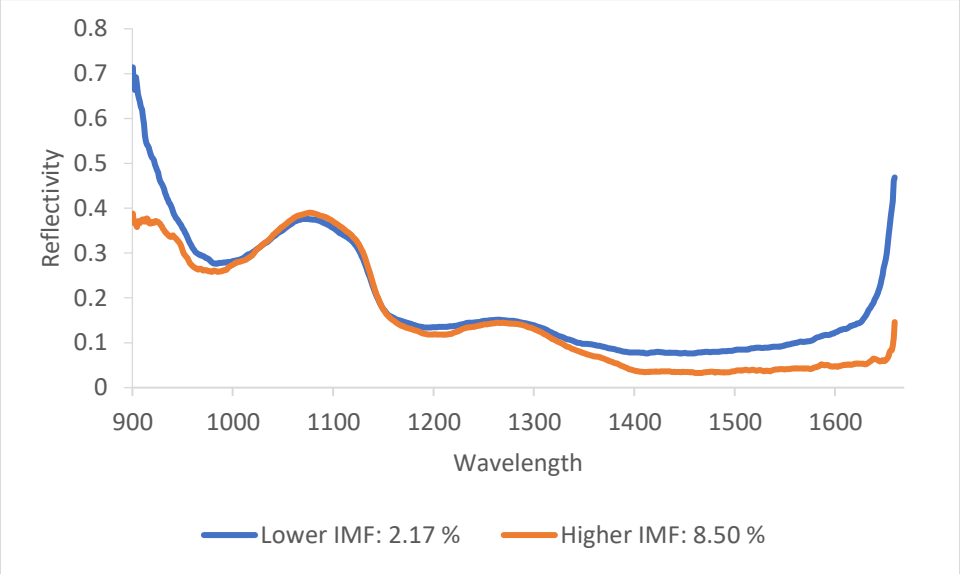


Figure 3.7: Averaged spectrum of 5 samples with highest fat and 5 samples with lowest fat at day 3 post-mortem in R mode. R, spectra collected with polarizer axis at 90 °angle to muscle fiber dir ection.



Tables:

Table 3.1: Mean, standard deviation (SD), minimum (Min) and maximum (Max) values of quality characteristics of beef LT muscle measured at day 3 post-mortem.

Characteristic	Mean	SD	Min	Max
L*	34.70	1.65	31.41	38.40
a*	19.04	1.75	15.36	23.47
b*	2.65	1.76	-1.27	6.39
Drip loss	1.68	1.19	0.15	5.40
Cooking Loss	16.63	2.76	9.36	22.65
WBSF	50.78	11.62	31.21	75.26
pH	5.61	0.16	5.11	6.08
IMF	4.60	2.03	1.97	10.01

Table 3.2: Mean, standard deviation (SD), minimum (Min) and maximum (Max) values of quality characteristics of beef LT muscle measured at day 12 post-mortem.

Characteristic	Mean	SD	Min	Max
L*	36.36	1.80	31.95	39.96
a*	21.10	1.09	19.11	23.31
b*	4.90	1.36	1.82	7.49
Drip loss	1.45	0.97	0.37	4.23
Cooking Loss	17.67	2.75	8.41	24.20
WBSF	35.33	7.22	19.98	50.67
pH	5.61	0.16	5.37	6.11
IMF	5.11	2.36	2.09	14.71

Table 3.3: Calibration and validation statistics of NIR PLS models at day 3 post-mortem (n=5).

Characteristic/Mode	Coefficient of Determination	N / Day 3[1]	P / Day 3[2]	R / Day 3[3]
L*	R^{2x}	0.85 ± 0.00^{a}	0.90 ± 0.00^{b}	0.90 ± 0.00^{b}
	r^{2y}	0.66 ± 0.03^{a}	0.76 ± 0.02^{b}	0.69 ± 0.10^{a}
a*	R^2	0.87 ± 0.00^{a}	0.96 ± 0.00^{b}	0.92 ± 0.01^{c}
	r^2	0.70 ± 0.02^{a}	0.83 ± 0.01^{b}	0.77 ± 0.01^{c}
b*	R^2	0.94 ± 0.00^{a}	0.89 ± 0.00^{b}	0.92 ± 0.00^{c}
	r^2	0.83 ± 0.01^{a}	0.81 ± 0.01^{b}	0.83 ± 0.01^{a}
Drip loss	R^2	0.86 ± 0.00^{a}	0.87 ± 0.01^{b}	0.89 ± 0.02^{c}
	r^2	0.70 ± 0.02^{a}	0.69 ± 0.03^{b}	0.74 ± 0.02^{c}
Cooking Loss	R^2	0.76 ± 0.02^{a}	0.92 ± 0.02^{b}	0.94 ± 0.00^{b}
	r^2	0.56 ± 0.02^{a}	0.80 ± 0.01^{b}	0.85 ± 0.01^{c}
WBSF	R^2	0.83 ± 0.00^{a}	0.81 ± 0.00^{b}	0.91 ± 0.01^{c}
	r^2	0.70 ± 0.04^{a}	0.59 ± 0.02^{b}	0.81 ± 0.01^{c}
pH	R^2	0.82 ± 0.00^{a}	0.90 ± 0.00^{b}	0.93 ± 0.00^{c}
	r^2	0.60 ± 0.05^{a}	0.78 ± 0.02^{b}	0.79 ± 0.03^{b}
IMF[4]	R^2	0.86 ± 0.01^{a}	0.91 ± 0.00^{b}	0.94 ± 0.00^{c}
	r^2	0.77 ± 0.01^{a}	0.81 ± 0.01^{b}	0.83 ± 0.00^{b}

[1]Spectra collected at day 3 post mortem with no polarizer.
[2]Spectra collected at day 3 post mortem with polarizer axis parallel to the muscle fiber direction.
[3]Spectra collected at day 3 post mortem with polarizer axis perpendicular to the muscle fiber direction.
[4] Intramuscular fat.
[x] Coefficient of determination of calibration.
[y] Coefficient of determination of prediction.
[a,b,c] Least squares means, within a column, lacking common superscript letters, differ according to analysis of variance ($P<0.05$).

Table 3.4: Calibration and Validation statistics of NIR PLS models at day 12 post-mortem (n=5).

Characteristic/Mode	Coefficient of Determination	N / Day 12[1]	P / Day 12[2]	R / Day 12[3]
L*	R^{2x}	0.83 ± 0.02^{a}	0.92 ± 0.005^{b}	0.88 ± 0.00^{c}
	r^{2y}	0.67 ± 0.01^{a}	0.82 ± 0.006^{b}	0.76 ± 0.01^{c}
a*	R^2	0.81 ± 0.00^{a}	0.88 ± 0.00^{b}	0.86 ± 0.01^{c}
	r^2	0.52 ± 0.02^{a}	0.62 ± 0.02^{b}	0.72 ± 0.03^{c}
b*	R^2	0.89 ± 0.00^{a}	0.88 ± 0.00^{b}	0.90 ± 0.00^{a}
	r^2	0.80 ± 0.01^{a}	0.81 ± 0.01^{a}	0.78 ± 0.01^{b}
Drip loss	R^2	0.76 ± 0.01^{a}	0.80 ± 0.00^{b}	0.86 ± 0.01^{c}
	r^2	0.66 ± 0.01^{a}	0.59 ± 0.05^{b}	0.73 ± 0.01^{c}
Cooking Loss	R^2	0.79 ± 0.00^{a}	0.86 ± 0.003^{b}	0.87 ± 0.00^{b}
	r^2	0.63 ± 0.04^{a}	0.61 ± 0.01^{a}	0.73 ± 0.02^{b}
WBSF	R^2	0.89 ± 0.00^{a}	0.83 ± 0.00^{b}	0.89 ± 0.00^{a}
	r^2	0.72 ± 0.00^{a}	0.63 ± 0.02^{b}	0.76 ± 0.01^{c}
pH	R^2	0.69 ± 0.02^{a}	0.85 ± 0.00^{b}	0.95 ± 0.00^{c}
	r^2	0.50 ± 0.02^{a}	0.65 ± 0.01^{b}	0.84 ± 0.02^{c}
IMF[4]	R^2	0.79 ± 0.01^{a}	0.90 ± 0.00^{b}	0.89 ± 0.01^{c}
	r^2	0.56 ± 0.05^{a}	0.78 ± 0.01^{b}	0.69 ± 0.02^{c}

[1]Spectra collected at day 12 post-mortem with no polarizer.
[2]Spectra collected at day 12 post-mortem with polarizer axis parallel to the muscle-fiber directio n.
[3]Spectra collected at day 12 post-mortem with polarizer axis perpendicular to the muscle fiber direction.
[4] Intramuscular fat.
[x] Coefficient of determination of calibration.
[y] Coefficient of determination of prediction.
[a,b,c] Least squares means, within a column, lacking common superscript letters, differ according to least square mean differences ($P<0.05$).

Table 3.5: Effect of NIR mode (N, P, R) on mean reflectivity of 47 samples at wavelengths 980 nm, 1074 nm, 1200 nm, 1274 nm, 1358 nm and 1450 nm.

Mode/Wavelength	980 nm	1074 nm	1200 nm	1274 nm	1358 nm	1450 nm
N	0.237 [a]	0.363 [a]	0.110 [a]	0.129 [a]	0.063 [a]	0.033 [a]
P	0.235 [a]	0.353 [a]	0.106 [b]	0.124 [b]	0.061 [a]	0.033 [a]
R	0.241 [a]	0.361 [a]	0.116 [c]	0.134 [c]	0.072 [b]	0.041 [b]
SEM	0.006	0.008	0.005	0.005	0.005	0.007
P value	0.15	0.47	<0.001	<0.001	<0.001	<0.001

[a,b,c] Least squares means, within a column, lacking common superscript letters, differ according to least square mean differences ($P<0.05$)($n=5$).

Table 3.6: Effect of NIR mode (N, P, R) on reflectivity of the five samples with greatest L* and five samples with smallest L* at wavelengths 980 nm, 1074 nm, 1200 nm, 1274 nm, 1358 nm and 1450 nm.

Mode/Wavelength	980 nm	1074 nm	1200 nm	1274 nm	1364 nm	1450 nm
N	0.233 [a]	0.353 [a]	0.112 [ab]	0.128 [ab]	0.063 [a]	0.037 [a]
P	0.232 [a]	0.340 [a]	0.105 [a]	0.119 [a]	0.059 [a]	0.036 [a]
R	0.240 [a]	0.353 [a]	0.117 [b]	0.132 [b]	0.071 [b]	0.046 [b]
SEM	0.018	0.013	0.01	0.009	0.009	0.008
P value	0.44	0.72	0.009	0.008	0.001	0.01

[a,b,c] Least squares means, within a column, lacking common superscript letters, differ according to least square mean differences ($P<0.05$)($n=5$).

Table 3.7: Effect of NIR mode (N, P, R) on reflectivity of the five samples with greatest a* and five samples with smallest a* at wavelengths 980 nm, 1074 nm, 1200 nm, 1274 nm, 1358 nm and 1450 nm.

Mode/Wavelength	980 nm	1074 nm	1200 nm	1274 nm	1364 nm	1450nm
N	0.244 [a]	0.376 [a]	0.113 [a]	0.134 [a]	0.062 [a]	0.032 [a]
P	0.250 [a]	0.375 [a]	0.114 [a]	0.134 [a]	0.067 [ab]	0.037 [a]
R	0.256 [a]	0.38 [a]	0.123 [a]	0.149 [b]	0.075 [b]	0.049 [b]
SEM	0.015	0.014	0.006	0.007	0.007	0.009
P value	0.15	0.44	0.05	0.001	0.04	<0.001

[a,b,c] Least squares means, within a column, lacking common superscript letters, differ according to least square mean differences (P<0.05). (n=5)

Table 3.8: Effect of NIR mode (N, P, R) on reflectivity of the five samples with greatest b* and five samples with smallest b* at wavelengths 980 nm, 1074 nm, 1200 nm, 1274 nm, 1358 nm and 1450 nm.

Mode/Wavelength	980 nm	1074 nm	1200 nm	1274 nm	1364 nm	1450nm
N	0.246 [a]	0.377 [a]	0.116 [ab]	0.140 [b]	0.067 [ab]	0.037 [ab]
P	0.241 [a]	0.368 [a]	0.112 [a]	0.130 [a]	0.062 [a]	0.033 [a]
R	0.246 [a]	0.376 [a]	0.120 [b]	0.143 [b]	0.071 [b]	0.044 [b]
SEM	0.023	0.025	0.011	0.011	0.009	0.01
P value	0.61	0.06	0.02	<0.001	0.04	0.03

[a,b,c] Least squares means, within a column, lacking common superscript letters, differ according to least square mean differences (P<0.05)(n=5).

Table 3.9: Effect of NIR mode (N, P, R) on reflectivity of the five most tender and five most tough muscles at wavelengths 980 nm, 1074 nm, 1200 nm, 1274 nm, 1358 nm and 1450 nm.

Mode/Wavelength	980 nm	1074 nm	1200 nm	1274 nm	1364 nm	1450 nm
N	0.246 [a]	0.373 [a]	0.113 [a]	0.135 [a]	0.063 [a]	0.037 [a]
P	0.246 [a]	0.363 [a]	0.112 [a]	0.132 [a]	0.067 [ab]	0.047 [b]
R	0.254 [a]	0.376 [a]	0.122 [b]	0.144 [b]	0.076 [b]	0.049 [b]
SEM	0.011	0.011	0.005	0.005	0.006	0.008
P value	0.35	0.66	0.006	0.006	0.006	0.011

[a,b,c] Least squares means, within a column, lacking common superscript letters, differ according to least square mean differences ($P<0.05$). (n=5)

Table 3.10: Effect of NIR mode (N, P, R) on reflectivity of the five samples with greatest intramuscular fat and five samples with smallest intramuscular fat at wavelengths 980 nm, 1074 nm, 1200 nm, 1274 nm, 1358 nm and 1450 nm.

Mode/Wavelength	980 nm	1074 nm	1200 nm	1274 nm	1364 nm	1450nm
N	0.252 [a]	0.379 [a]	0.113 [a]	0.134 [a]	0.063 [a]	0.033 [a]
P	0.257 [a]	0.373 [a]	0.116 [ab]	0.136 [a]	0.067 [ab]	0.036 [a]
R	0.260 [a]	0.383 [a]	0.125 [b]	0.146 [b]	0.077 [b]	0.050 [b]
SEM	0.012	0.012	0.007	0.007	0.009	0.012
P value	0.54	0.14	0.01	0.008	0.009	0.003

[a,b,c] Least squares means, within a column, lacking common superscript letters, differ according to least square mean differences ($P<0.05$)(n=5).

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4. General Summary

The quest for an objective non-invasive method to predict meat quality characteristics has led to exploring the potential for using NIR spectra to predict these characteristics The use of near infrared (NIR) spectroscopy to predict the proximate composition of meat (intramuscular moisture, fat, and protein) has been already commercialized and recognised by AOAC, which has approved a method using NIR spectroscopy in transmittance mode and artificial neural network prediction models for the proximate composition of meat (Anderson, 2007). However, few studies have been successful in predicting the technological characteristics of meat. Description of meat technological characteristics, which include muscle colour, drip loss and especially meat tenderness, is critical because these characteristics have the potential to dictate the consumer acceptance of meat and influence their repurchasing decision. The objective of this thesis was to explore the application of polarization in NIR spectroscopy to predict meat toughness, muscle colour variables, drip loss and intramuscular pH and fat. As 90% of feedlot-finished slaughtering cattle in North America have been subjected to at least one implant in their life, it is important to incorporate animals subjected to growth promotants in a calibration data set to ensure description of beef from these production systems is possible using polarized NIR spectroscopy as growth promotants are reported to introduce changes in muscle structures of animals. Limited variation in the reference data used to develop NIR prediction models is one of the major reasons for poor accuracy of NIR algorithms. As growth promotants are recorded in literature as able to induce toughness in beef and to alter other meat quality characteristics, they were considered to be a viable way of introducing variation into the reference data. Chapter 1 examined the effect of growth promotants, both hormonal growth promotants (HGP) and β adrenergic agonists (ractopamine (RAC)) feed supplements, as well as selection for residual feed

intake (RFI) on meat toughness and other meat quality characteristics. Results suggested that there was no effect of RFI or RAC on meat toughness. HGP increased beef toughness by more than 10 N at day 3 post-mortem but the carcasses subjected to HGP had a greater response to post-mortem aging as the meat toughness of HGP steers was statistically similar to that of control steers at day 12 post-mortem. As aging tenderizes the meat due to the action of proteolytic enzymes and the effect of HGP on WBSF ceases, the range of reference WBSF value was decreased at day 12 post-mortem which then decreased the coefficient of correlation for the WBSF reference data on day 12 post mortem. Post-mortem aging also had the largest effect among the treatments (RFI, HGP, RAC) on meat colour. There was an increase in the means of the colour variables L*, a* and b* values at day 12 post-mortem when compared to day 3 post-mortem and, as a result, the standard deviation of the reference L* value increased with aging, of a* decreased with aging and of b* did not change. This change in the standard deviation of the reference data can be seen in the NIR calibration created in chapter 3 which show that the predictability of NIR calibration model increased for L*, decreased for a* and was similar for b* at day 12 post-mortem when compared to the r^2 value of the prediction models at day 3 post-mortem. Although there have been several attempts to predict technological characteristics using NIR spectroscopy, no research has included meat from animals treated with growth promotants which in this study has helped to increase the range of reference WBSF data and may be one of the reasons why moderate r^2 of validation for WBSF were achieved. In the third chapter, the possibility of using polarized light during NIR spectroscopy and the role of the degree of polarization on the predictability of NIR models were also investigated. No published research to date has explored this area. It was found that the angle of polarization with reference to the muscle fiber direction has a significant effect on the calibration models. Spectra collected when

the polarizer axis was perpendicular to the muscle fiber direction (R mode) at day 3 post-mortem gave the best validation r^2 for WBSF, drip loss, and cooking loss. Colour measurements L* and a* were best predicted when the polarizer axis was parallel to the muscle fiber direction (P mode). The IMF and pH were best predicted with a polarizer, with no effect of the angle of polarization. The angle of polarization may affect the predictability of NIR calibration by affecting reflectivity. It was found that at 1200, 1274, 1358 and 1450 nm, the reflectivity was greater in the R configuration than in the P configuration and/or when no polarization was used. In this study, NIR models were created using reflectivity data rather than absorbance data, which is a practice that most other researchers in meat science do not use. Taking reflectivity (reflectance of the sample divided by reflectance from the reference surface (Teflon^{TM} disk)) instead of using direct reflectance might have benefited the accuracy of the models by normalization as it accounts for the daily changes in the machine and in the environment.

There were some aspects of the field of meat quality prediction using light spectroscopy that were not investigated in this thesis. In Chapter 1, the animals were selected for inclusion in the study based on their actual residual feed intake rather than on genetic selection for it, but breeding for RFI may have an effect on meat quality, especially beef toughness if the animals are selectively bred for 4-5 generation. Although in this experiment RFI did not interact with HGP and RAC to have a deleterious effect on meat quality, this warrants further research, and it should be explored using cattle selectively bred for RFI for 4-5 generations. Also, this thesis has used partial least square regression (PLSR) for calibration development, but artificial neural network analyses could be used to investigate if it can predict the meat quality characteristics with greater accuracy than PLSR. The success of NIR calibration depends on the precision of the

reference method, and to complicate this, the WBSF method has been reported to lack precision. Slice shear force is another method to assess toughness of meat reported to have a lower coefficient of variation than WBSF and so, in turn, may produce more repeatable estimation of shear force. A more repeatable estimation of shear force may be more strongly correlated with sensory analysis of meat tenderness than WBSF. Slice shear force is worthy of investigation and incorporation into future experimentation to minimize error due to the lack of precision of the reference method.

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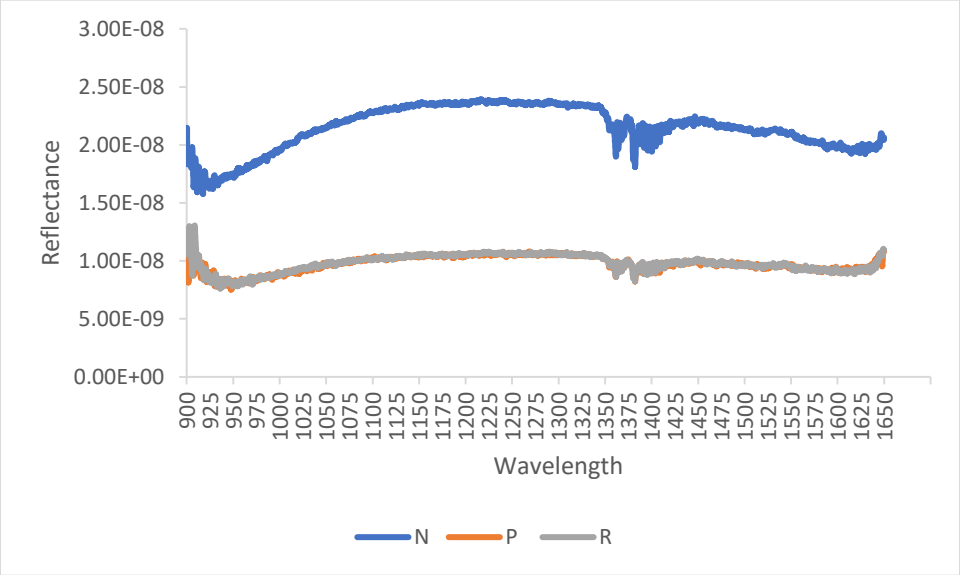
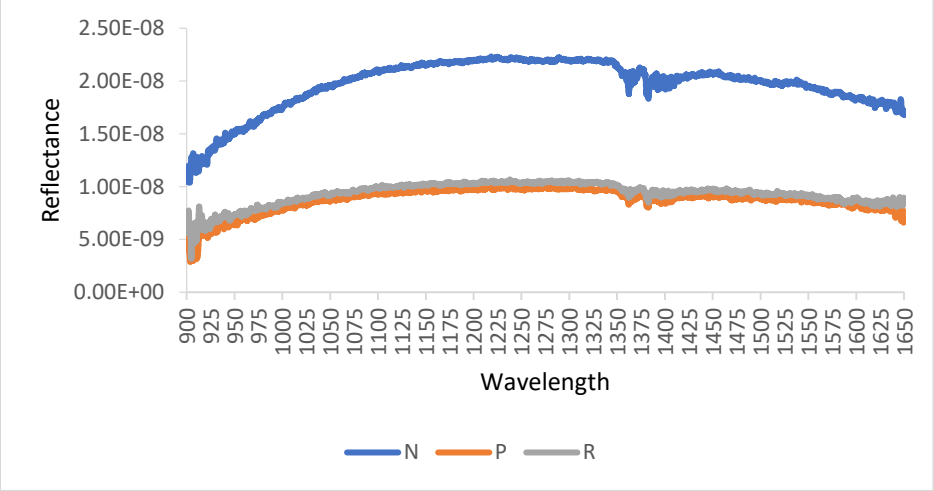
Appendix - Reflectance from Teflon™ disk without polarizer (N), with polarizer when the polarizer axis was parallel to the muscle fiber direction (P), with polarizer when the polarizer axis was perpendicular to the muscle fiber direction (R)

Figure 4.1 Reflectance from Teflon™ at day 3 post-mortem for Kill 1 data set.



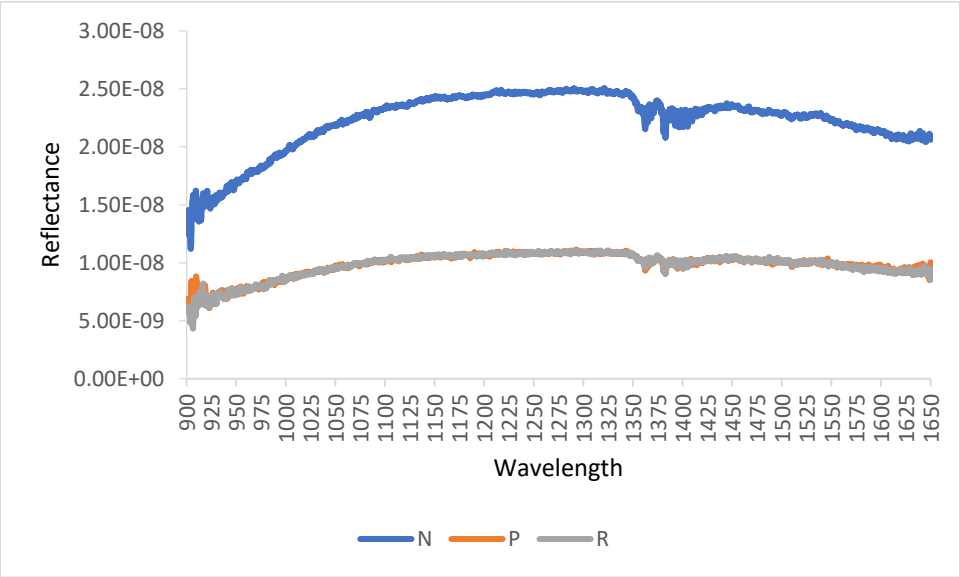
N, spectra collected with no polarizer; P, spectra collected with polarizer axis parallel to the muscle fiber direction; R, spectra collected with polarizer axis at 90 °angle to muscle fiber direction.

Figure 4.2 Reflectance from Teflon™ disk at day 3 post-mortem for Kill 2 data set.



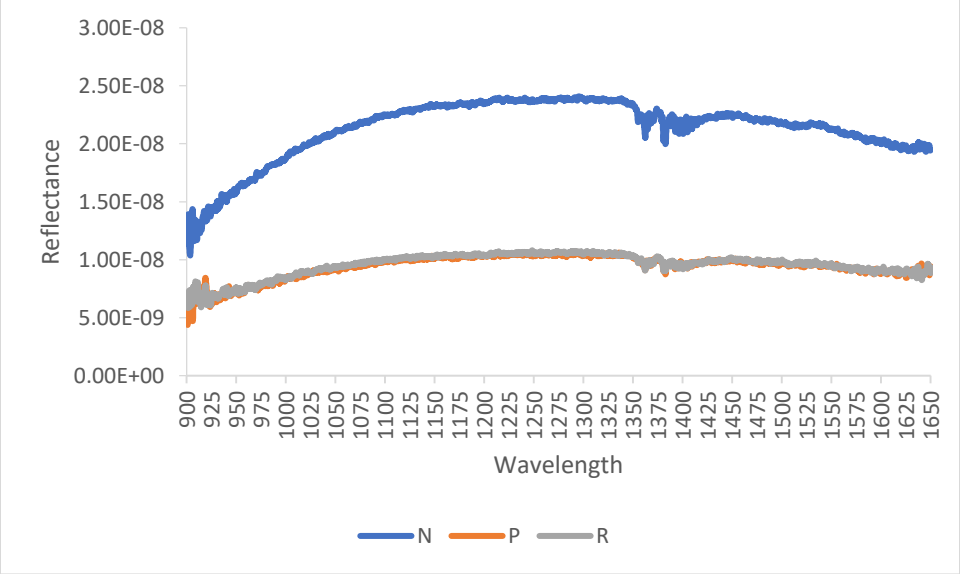
N, spectra collected with no polarizer; P, spectra collected with polarizer axis parallel to the muscle fiber direction; R, spectra collected with polarizer axis at 90 °angle to muscle fiber direction.

Figure 4.3 Reflectance from Teflon™ disk at day 3 post-mortem for Kill 3 data set.



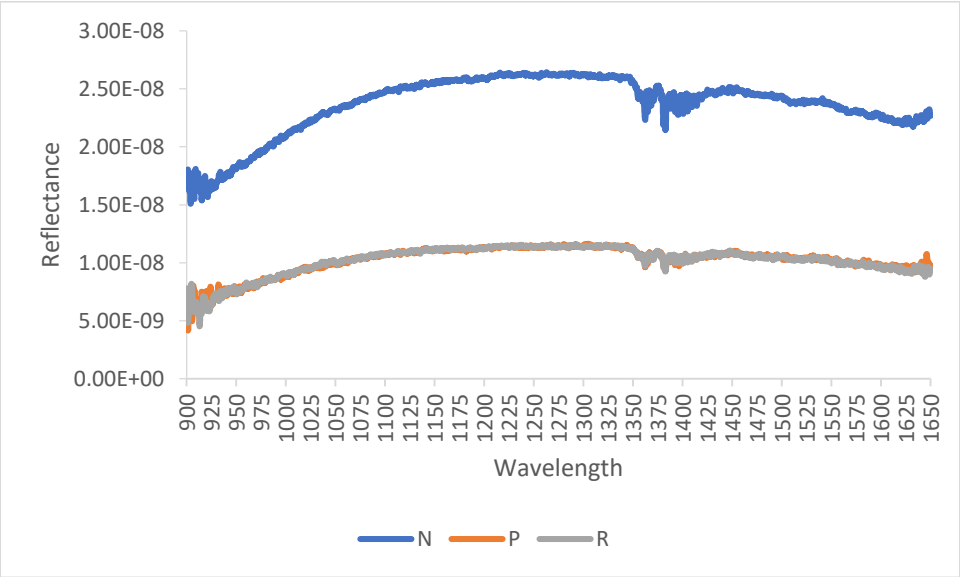
N, spectra collected with no polarizer; P, spectra collected with polarizer axis parallel to the muscle fiber direction; R, spectra collected with polarizer axis at 90 °angle to muscle fiber direction.

Figure 4.4 Reflectance from Teflon™ disk at day 3 post-mortem for Kill 4 data set.



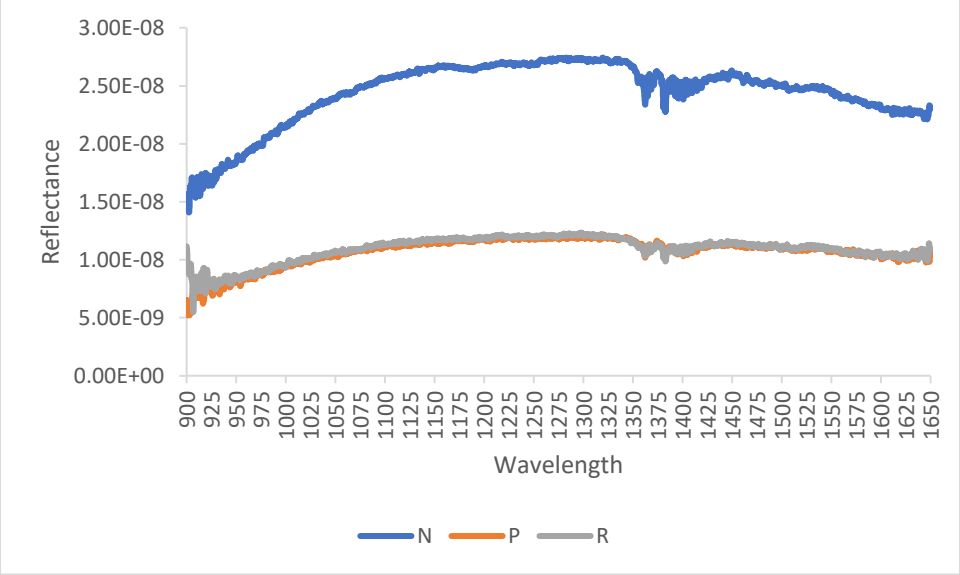
N, spectra collected with no polarizer; P, spectra collected with polarizer axis parallel to the muscle fiber direction; R, spectra collected with polarizer axis at 90 °angle to muscle fiber direction.

Figure 4.5 Reflectance from Teflon™ disk at day 3 post-mortem for Kill 5 data set.



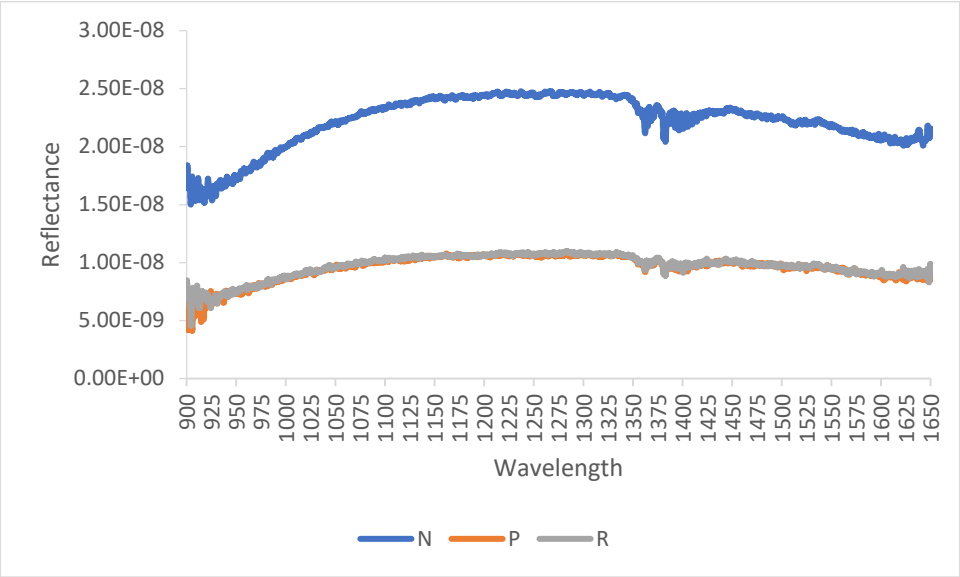
N, spectra collected with no polarizer; P, spectra collected with polarizer axis parallel to the muscle fiber direction; R, spectra collected with polarizer axis at 90 °angle to muscle fiber direction.

Figure 4.6 Reflectance from Teflon™ disk at day 3 post-mortem for Kill 6 data set.



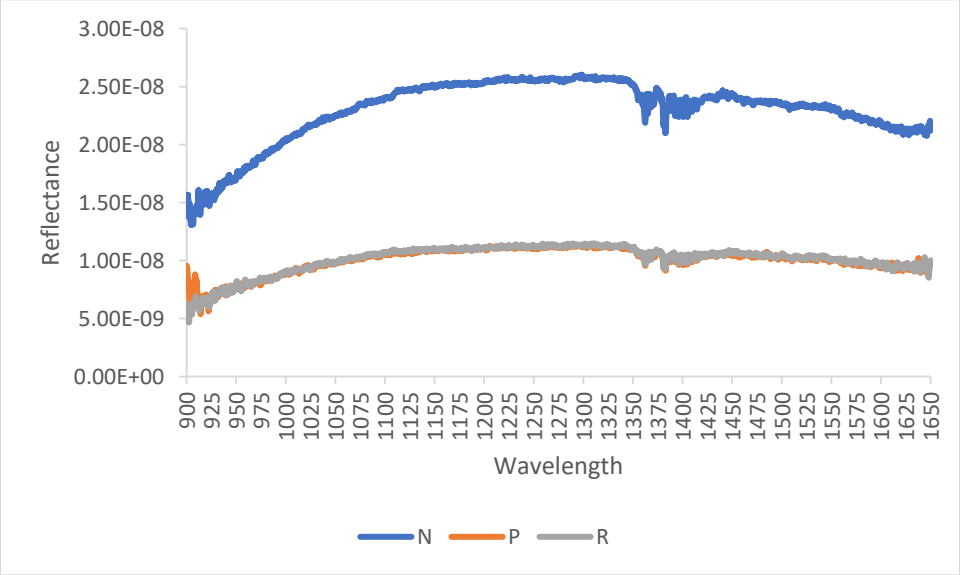
N, spectra collected with no polarizer; P, spectra collected with polarizer axis parallel to the muscle fiber direction; R, spectra collected with polarizer axis at 90 °angle to muscle fiber direction.

Figure 4.7 Reflectance from Teflon™ disk at day 12 post-mortem for Kill 1 data set.



N, spectra collected with no polarizer; P, spectra collected with polarizer axis parallel to the muscle fiber direction; R, spectra collected with polarizer axis at 90 °angle to muscle fiber direction.

Figure 4.8 Reflectance from Teflon™ disk at day 12 post-mortem for Kill 6 data set.



N, spectra collected with no polarizer; P, spectra collected with polarizer axis parallel to the muscle fiber direction; R, spectra collected with polarizer axis at 90 °angle to muscle fiber direction.