Trained panel sensory evaluation of *m. longissimus thoracis*, *m. gluteus medius* and *m. semimembranosus* from Angus crossbred steers: the influence of hormonal growth promotants, ractopamine hydrochloride, and selection for high or low residual feed intake.

by

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Abstract

Selection of cattle for residual feed intake (RFI) does not affect rate of weight gain; rather, low RFI cattle consume less feed than high RFI animals to attain a similar final body weight and are thus considered feed efficient. Reducing production costs by selecting low RFI cattle and incorporating hormonal growth promotants (HGP) or beta-adrenergic agonists (BA) into beef production programs will only be beneficial to the beef industry if meat quality is not adversely affected. Trained sensory panelists evaluated the meat quality characteristics of *m. longissimus thoracis* (LT), *m. gluteus medius* (GM), and *m. semimembranosus* (SM) from carcasses of Angus crossbred steers treated with HGP and/or BA and selected for high or low RFI. Forty-eight Angus crossbred steers, 21 high RFI and 27 low RFI, were either implanted twice with HGP (treated) or not (control) and received either ractopamine at 200 mg/head/day for the last 28 days of finishing (treated) or not (control) in a 2 x 2 x 2 factorial design. Half of each muscle, balanced for position within the muscle, was aged under vacuum in polypropylene bags for either 3 or 12 days post-mortem before assessment for sensory characteristics. Overall tenderness scores for all the samples were rated as tender, but aged samples were rated highest for overall tenderness. The results further showed that steaks from animals that were treated with HGP were the toughest, while steaks from animals that were either treated with ractopamine hydrochloride (RAC) or not treated produced the most tender beef regardless of muscle type. A similar relationship was observed for juiciness with HGP steaks the least juicy by the panelists while steak from steers with no treatment or only RAC were the juiciest.

Preface

This thesis describes one study: the use of trained sensory panelists to evaluate the meat quality characteristics of *m. longissimus thoracis*, *m. gluteus medius* and *m. semimembranosus* from Angus crossbred steers selected for high or low residual feed intake and treated with hormonal growth promotants, the beta adrenergic agonist ractopamine hydrochloride, or both, or none. Beef cattle used were reared at University of Alberta Kinsella cattle herd according to Kinsella animal care protocol: AUP00001801 and slaughtered at "Love's Custom Meats" a provincial abattoir located in Vegreville, Alberta. The samples were stored frozen at Agri-Food Discovery Place (AFDP) at the University of Alberta South Campus until needed. This study received research ethics approval from the University of Alberta Research Ethics Board, under the complete study title: "Effect of steroids and ractopamine in beef steers on trained sensory panel characterization of the eating quality of one bovine muscle", Pro00073730, on 22 February 2018, with two further amendments to accommodate testing of the other two muscles. The experimental design, panel recruitment, data collection and data analyses are my original work, with the assistance of Dr. Heather Bruce, Dr. Wendy Wismer and some colleagues.

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Abbreviations

ADG	Average daily gain
ADI	Acceptable daily intake
AGP	Antimicrobial growth promoters
APHA	American Public Health Association
AMSA	American Meat Science Association
ANOVA	Analysis of variance
β	Beta
β-ARs	Beta-adrenergic receptors
BA	Beta adrenergic / agonist
BW	Body weight
CNN(A)	Control RFI, no steroids, no ractopamine (Aged)
CNY(A)	Control RFI, no steroids, yes ractopamine (Aged)
CYN(A)	Control RFI, yes steroids, no ractopamine (Aged)
CYY(A)	Control RFI, yes steroids, yes ractopamine (Aged)
DMI	Dry matter intake
ENN(A)	Efficient RFI, no steroids, no ractopamine (Aged)
ENY(A)	Efficient RFI, no steroids, yes ractopamine (Aged)
EYN(A)	Efficient RFI, yes steroids, no ractopamine (Aged)
EYY(A)	Efficient RFI, yes steroids, yes ractopamine (Aged)
EU	European Union
FAO	Food and Agriculture Organization

FCR	Feed conversion ratio
FDA	Food and Drug Administration
GDP	Gross domestic product
GM	m.gluteus medius
GOC	Government of Canada
GP	Growth promoters
HCW	Hot carcass weight
HGP	Hormonal growth promoter
IGF-1	Growth factors
IMF	Intramuscular fat
JECFA	Joint Expert Committee on Food Additives
LT	m.longissimus thoracis
M.	Muscle
MGA	Melengestrol acetate
MRL	Maximum residual level
MT	Metric tonne
NOAEL	No observed adverse effect level
QDA	Quantitative Descriptive Analysis
RAC	Ractopamine hydrochloride
RFI	Residual feed intake
RNA	Ribonucleic acid
SM	m.semimembranosus

SOP	Standard operating procedure
ST	Somatropin
STE	Steroid
US	United States
WBSF	Warner-Bratzler shear force
WHO	World Health Organization

CHAPTER 1: Introduction and Literature Review

The beef industry is an important industry in Canada with a population of 3.83 million beef cattle as of January 2017 (Canadian Cattlemen Association (CCA) 2017). The beef industry contributes $17.2 billion to the Canadian gross domestic product (GDP), and Canada exports about 47% of its annual production, which is approximated at 1.3 million tonnes (MT) (CCA 2017). The beef industry in Canada therefore strives for continuous improvement in the yield, standard and quality of the beef produced.

Beef quality is a broad term. It encompasses all the essential criteria required for beef to be fit for consumption, processing and storage. Aspects of meat quality include but are not limited to its nutritional properties, its safety, and its sensory properties (Sevi et al. 2016; Špehar and Žgur, 2008; Dagne and Ameha, 2017; Maltin et al. 2003). While these aspects undoubtedly are important for consumer acceptance, the sensory properties play a crucial role. Factors such as the degrees of tenderness, flavour and juiciness of beef samples greatly influence consumer choice of beef (Špehar and Žgur, 2008; Smith and Carpenter, 1974; Reicks et al. 2011) with tenderness being the most important factor (Jeremiah 1982; Huffman et al. 1996; Miller et al. 2001). As evidence of this, studies have shown that consumers are willing to pay more for a guaranteed tender beef cut (Calkins and Sulivan, 2007; Hanagriff et al. 2009; Špehar and Žgur, 2008).

Variation in beef tenderness is a major challenge in the beef industry (Špehar and Žgur, 2008) and a potential cause of revenue loss. Therefore, to maximize revenue obtained per animal, it is important to ensure that the maximum amount of beef is obtained per cattle head and that these cuts are within the acceptable tenderness range for consumers. Cattle producers are therefore continuously searching for the means to improve both beef yield and quality, and have explored genomics, post-rigor processing activities and growth enhancement of cattle with growth promoters such as hormones and beta agonists (Colle et al. 2018; Aalhus et al. 1999; Cassar-Malek and Picard, 2016).

The use of growth promoters is a promising option for improving carcass quality and yield as they have been shown to improve muscle mass (Johnson et al. 2014), thus increasing the amount of beef obtained per cattle head. Studies on their effect on sensory and palatability properties of the resulting beef have yielded contrasting results. Fernández-Dueñas et al. (2008), Avendano-

Reyes et al. (2006), and Barham et al. (2003) found that beef tenderness decreased with HGP use. Lean et al. (2018), however, noted that using multiple HGP did not have any negative effect on the sensory tenderness ratings, and that flavour and juiciness were not associated with HGP use. Boler et al. (2012) also noted that using growth promoters had a negative effect on meat tenderness.

Consumers have also raised concerns about the use of growth promoters in growing cattle as people care about the food they eat and want to be certain that what they eat is raised with integrity, is safe and is best for them (Ellison et al. 2017). Paris et al. (2006) concluded that edible tissue from veal calves, heifers and steers treated with HGP had progesterone concentrations that were not different than tissues from non-treated animals. In Canada, six hormonal implants are approved for use (Government of Canada (GOC), 2012; https://www.canada.ca/en/health-canada/services/drugs-health-products/veterinary-drugs/factsheets-faq/hormonal-growth-promoters.html). In the USA, 38 implants are approved for use in cattle (Food and Drug Administration (FDA), 2013; (https://www.fda.gov/AnimalVeterinary/SafetyHealth/ProductSafetyInformation/ucm055436.htm). Two beta agonists (ractopamine hydrochloride and zilpaterol hydrochloride) can be used in the USA and other countries including Brazil, Canada and China (Dilger, 2015). However, only ractopamine hydrochloride is used presently in North America as production of Zilmax™, (the tradename for zilpaterol hydrochloride) has been voluntarily suspended (Allen, 2014). While steroids (hormonal implants) have been used for over 50 years in cattle production, the use of beta agonists is recent, and the effects are still not fully understood.

Selection of cattle for residual feed intake (RFI) has also recently been identified as a mechanism for increasing animal feed efficiency and reducing the cost of beef production. Residual feed intake is defined as the difference between an animal's actual feed intake and its expected feed requirements for maintenance and growth (Koch et al. 1963). Efficient animals eat less than expected and have a negative or low RFI, while inefficient animals eat more than expected and have a positive or high RFI. RFI is the preferred method of estimating cattle efficiency because it uses energy intake and energy requirements to calculate feed utilization and is independent of body weight and size (Sainz et al. 2004).

To date, no literature appears to be available that explores the effects of interactions between RFI and growth promoting technologies such as HGP and beta-adrenergic agonists on the sensory properties of beef. This work seeks to investigate the effects on the sensory properties of beef from cattle selected for high or low RFI and the interaction of RFI with ractopamine and/or steroids. To fully appreciate the impact of these treatments on beef quality, we must understand how beef quality is defined and how these technologies and other factors may affect it, and this will be the focus of this review.

1.1 Beef Sensory Quality

Meat quality can be broadly classified into three major aspects; safety, nutritional and palatability properties (Dagne and Ameha 2017; Maltin et al. 2003) but should also include appearance, as consumer purchasing decisions are influenced heavily by meat colour (Holman et al. 2017). The beef industry is consumer driven and the palatability of a beef cut greatly influences the repurchasing decision of a consumer (Miller et al. 2001). The palatability of a beef cut is the eating quality of that cut and it is determined by its tenderness, juiciness, and flavor (Miller et al. 2001). However, as much as beef is demanded by consumers, a major source of dissatisfaction arises from the variation in these palatability properties, especially that of beef tenderness (Tian et al. 2013; Maltin et al. 2003). Factors such as chronological age of the animal (Špehar and Žgur, 2008), breed/genetics (Dagne and Ameha, 2017), muscle type (Guerrero et al. 2013), muscle function and location (Calkin and Sullivan,2007), post mortem ageing conditions (Tian et al. 2013, Smith et al. 1978), environmental factors (Khan et al. 2016), growth promoting treatments (Listrat et al. 2016), carcass composition (marbling) (Avendano-Reyes et al 2006), pre-slaughter handling (Fernández-Dueñas et al. 2008), sex, and diet (Juarez et al. 2011) can contribute to this variation.

1.1.1 Tenderness

This is the most important palatability property of a beef cut and a major deciding factor of North American and Ethiopian consumer willingness to repurchase (Dagne and Ameha, 2017; Maltin et al. 2003; Smith et al. 1978; Calkin and Sullivan, 2007). The most important factors reported to influence beef tenderness are animal age, muscle location, and length of post mortem ageing (Koohmaraie, 1994, Smith et al. 1978). The older an animal is, the tougher the beef is likely to be; therefore, the greatest tenderness and beef quality is obtained from an animal less

than 36 months old (Dagne and Ameha, 2017). Muscles used for locomotion are tougher than support muscles (Calkins and Sullivan, 2007) and aged carcasses usually produce muscles that are more tender than they were immediately post mortem (Khan et al. 2016). Breed and genetics also can play a role in tenderness of beef, with *Bos indicus* breeds such as Brahman producing beef that is often tougher than beef from cattle of *Bos taurus* genetics due to a reduced level of proteolytic degradation of myofibrillar proteins in the *Bos indicus* cattle muscles (Pereira et al. 2015). In general, variation in meat tenderness is greater within breeds than among breeds. (Špehar and Žgur, 2008).

1.1.2 Juiciness

The descriptor "juiciness" refers to the amount of juice released from beef during chewing (Dagne and Ameha, 2017). Although priority is given to tenderness, juiciness is also an important factor that influences consumer purchasing decisions (Juarez et al. 2011). Marbling plays an important role in the juiciness of a beef cut, as the perception of juiciness has been observed to increase with intramuscular fat (IMF), although the appearance of visible fat streaks on an uncooked beef cut could be a deterrent to a potential consumer (Maltin et al. 2003; Juarez et al. 2011).

1.1.3 Flavour

Flavour is the sensation perceived as a result of the interaction between the aromatic compounds and receptors within the nasal region and those in contact with the tongue during the chewing process. It is also an important property of beef and it is influenced by factors such as animal diet, ageing conditions, and breed (Dagne and Ameha, 2017; Khan et al. 2015).

1.1.4 Post mortem Ageing

Ageing is the process by which beef is stored or conditioned to improve its palatability (Kahraman and Gurbuz, 2018; Khan et al. 2016). This is achieved by storing beef in cold conditions for a period ranging from days to weeks to promote the breakdown of the muscle sarcomeric structure and the development of flavour by the action of endogenous enzymes. There is an array of evidence in the literature that ageing improves palatability properties such as tenderness, flavour and juiciness of beef. It also suggests that as the length of the ageing time

increases, the greater the increase observed in these properties (Khan et al. 2016; Dashdorj et al. 2016).

The changes in flavour, water holding capacity and tenderness observed in aged beef have been extensively studied and reported in the literature (Calkin and Sullivan, 2007; Tian et al. 2013; Dashdorj et al. 2016; Smith et al. 1978; Khan et al. 2016). These changes have been attributed to the actions of endogenous hydrolase enzymes. Calcium dependent enzymes, the calpains (μ-calpain, m-calpain), along with their inhibitor calpastatin are the major proteins involved in the proteolysis of myofibrillar proteins during the post mortem period, degrading the muscles and producing flavour peptides and free amino acids. The hydrolysis of fats, carbohydrates and ribonucleotides had also been reported to contribute to flavour development (Aaslyng and Meinert 2017; Koutsidis et al. 2008). Carbohydrates can be reduced to sugars, fats to aromatic fatty acids, and ribonucleotides to inositol monophosphate (IMP), guanidine monophosphate (GMP), inosine, and hypoxanthine (Mottram. 1998; Khan et al. 2016; Krahaman and Gurbuz, 2018).

Generally, there are two types of post mortem ageing; wet and dry ageing. Both methods seek to serve the same purpose but differ in the conditions of ageing. Dry ageing was the earliest method of ageing beef and involves large chunks of beef being suspended on hooks and kept in cold storage without protective packaging for a period. On the other hand, in vacuum/wet ageing, beef cuts are stored under vacuum in a moisture impermeable bag in cold storage (Ahnström et al. 2006; Khan et al. 2016; Krahaman and Gurbuz, 2018). Wet ageing is an economically favorable option as it provides packaging for transportation and increases yield by reducing product lost to the trimming and evaporation associated with dry ageing. Flavour obtained from wet aged beef has been characterized as metallic and bloody while dry ageing results in beef cuts with superior beefy or roasted flavour owing to concentration of flavour precursors and compounds such as aromatic fatty acid as moisture is lost from exposed areas (Khan et al. 2016). As such, dry-aged beef is priced as a premium product (Khan et al. 2016; Dashdorj et al. 2016). A more recent method is the use of a dry ageing bag which has a high vapor transmission rate thereby allowing the loss of moisture but mitigating microbial contamination, weight and trim loss associated with dry ageing while attaining flavour properties like dry aged beef (Dashdorj et al. 2016; Ahnström et al. 2006; Khan et al. 2016)

Conditions necessary to ensure proper dry ageing include proper storage temperatures (not below -2 ^{o}C), adequate air velocity, relative humidity and the number of days. Ageing conditions should be at temperatures from 0-3°C and 70-85% humidity (RH), with an air velocity of 0.2 to 0.5 m/s for 1 to 5 weeks although little change in meat quality characteristics is noted after 14 days of ageing (Krahaman and Gurbuz, 2018). Dashdorj et al. (2016) recommended storage conditions of 0-4°C, RH of 61-85% and an air flow range of 0.5–2 m/s for 28-55 days.

1.2 Evaluation of beef tenderness using Warner-Bratzler shear force

Beef tenderness is an important and highly varied aspect of beef quality. Toughness of meat can be evaluated objectively with a mechanical apparatus such as the Warner-Bratzler shear force (WBSF) machine (Destefanis et al. 2008; Silva et al. 2015) or by using a trained sensory panel, which can evaluate both tenderness or toughness objectively (Caine et al. 2003). Tenderness can also be evaluated subjectively using a consumer panel, with consumer panels usually used to determine the acceptability of a product (Sitz et al. 2005). When measured mechanically, toughness not tenderness is measured as the force required to cut through a piece of meat perpendicular to the muscle fiber direction, and it is usually quantified in either kg or Newtons (Purchas, 2014). Different attempts have been made to design instruments to quantify the force needed in tearing, biting, compressing and stretching the meat to give a prediction of the tenderness rating obtained from sensory panelist. The most common method for objectively measuring beef toughness is the use of the single blade shear test. The idea to use a steel blade to slide through a sample to measure the amount of force needed to shear the meat sample was first demonstrated by K.F. Warner in the 1920's, and was then modified by L.J. Bratzler to increase the test accuracy by standardizing the blade thickness, shape and speed (Zamarripa, 2014; Destefanis et al. 2008; Juarez et al. 2011; Silva et al. 2017). The Warner-Bratzler shear force (WBSF) device has a simple instrumental design that measures the force needed to shear across entire muscle fibers (Silva et al. 2015). According to Voisey and Larmond (1974), the specifications of instrument design given by Bratzler are: "A steel blade 1.016 mm (0.04 in) thick is moved through a slot that clears it by 0.127 mm (0.005 in) at a rate of 22.86 cm/min (9.0 in/min). This cuts a meat sample placed in a triangular hole in the blade. The maximum force on the blade is shown on a spring scale and used as a tenderness index. The hole is made by circumscribing an equilateral triangle about a circle 25.4 mm (1.0 in) diameter. The edges of the hole are radiused at 0.508 mm (0.02 in)".

In the WBSF method, a steak sample is cooked to an internal temperature of 71°C and cooled to a consistent temperature. A minimum of six 1.27 cm round cross-section cores are cut from the steak and the instrument with cross head speed of 200–250 mm/min is used to completely shear cores perpendicular to the muscle fiber orientation (Silva et al. 2015; AMSA, 2016). Due to the difficulty in obtaining a uniform round cross-section, several researchers have used square cross-sections which are easy to cut, highly uniform and facilitate easy recognition of muscle fiber orientation. An experiment on the comparison of the two methods showed high correlation between the two, asserting the possibility of the use of square cross-sections in place of round cross-sections whilst obtaining accurate data (Silva et al. 2015).

Three major factors have been reported to affect the accuracy of the WBSF measurements and these are cooking method and end-point temperature, steak and core orientation, and core orientation with respect to muscle fibers, the latter of which has been noted to have the largest potential impact on WBSF (Silva et al. 2015; Silva et al. 2017). The WBSF values obtained for beef toughness have been found to have varied correlation with consumer tenderness ratings (Voisey and Larmond, 1974). Several experiments have been performed utilizing trained panelist to establish threshold values of WBSF for tenderness acceptability. Values obtained within the range of < 42.87 N and > 52.68 N corresponded to consumer ratings for tender and tough beef respectively (Destefanis et al. 2008).

1.3 The structure of muscle

There are generally three classes of muscles: skeletal; cardiac; and smooth muscles. The muscle mass of livestock that produce human food represents 35 to 60% of their body weight (Listrat et al. 2016). The skeletal muscles are composed primarily of muscle fibers (about 90%), with connective and adipose tissues cumulatively accounting for about 10% of the total muscle (Listrat et al. 2016). Muscle fibers are long, multinucleated, spindle-shaped cells about 10 - 100 micrometers diameter. The muscle fiber is made up of myofibrils, which are series of sarcomeres, the sarcomere being the functional unit of muscle contraction consisting of the thick and thin filaments. The sarcomere consists primarily of the proteins actin (thin filament) and myosin (thick filament, 65%) and also tropomyosin and troponin T, I and C proteins (Listrat et al. 2016; Calkins and Sullivan, 2007). The connective tissue in the skeletal muscle is divided into the endomysium, perimysium and epimysium. Endomysium is connective tissue that surrounds each individual muscle fiber, perimysium is connective tissue that surrounds bundles

of muscle fibers and epimysium is connective tissue that surrounds the whole muscle. Each level of connective tissue consists of an extracellular matrix of collagen fibers enclosed with proteoglycans. The collagens are fibrous proteins with a helical structure consisting of three polypeptide chains wrapped around each other to form a triple helix (Listrat et al. 2016). These main components of muscles have been shown to have effects on the sensory quality of beef, especially beef tenderness (Lana and Zolla, 2016).

The ease of proteolytic degradation of actomyosin (formed by the combination of actin and myosin in the muscle fibers) and the sarcomere length of the muscle fiber have been reported to influence the tenderness of beef. Sarcomere length is affected by the muscle position and temperature during rigor mortis. A relaxed muscle has a long sarcomere length (> 2 μm) and is more tender than a muscle with contracted sarcomeres (<2 μm) (Calkins and Sullivan, 2007).

The quantity, heat-induced solubility (Calkins and Sullivan, 2007), composition, structure, organization and size of connective tissues determine the beef grain/ texture and subsequently the tenderness (Listrat et al. 2016). Muscles involved in locomotion have large amounts of connective tissue and as such have decreased tenderness whereas muscles of support are tender due to lack of consistent use (White, 2012). Also, the intermolecular cross-links that occur within collagen are responsible for its resilient tensile strength and heat stability. Heating results in the partial solubility of collagen by changing it into gelatin. The insoluble component that remains after the heating process is the most stable, covalently bonded mature collagen commonly found in the mature animal, as an increased percentage of soluble cross-links are replaced by heat stable cross-links with time, decreasing tenderness (Calkins and Sullivan, 2007; White, 2012).

Adipose tissue also plays a role in determining the degree of tenderness of beef by lowering the bulk myofibrillar density through dilution of the protein and providing lubrication between muscle fibers to reduce the amount of force needed to cut the meat (Calkins and Sullivan, 2007).

1.4 Growth Promoters

In broad terms, growth promoters are substances administered to animals to improve their feed utilization and to enhance growth. They are grouped into hormonal anabolic implants (both estrogenic and androgenic), bovine somatotropin, feed additives, repartitioning agents (beta

adrenergic agonists) and probiotics (Herago and Agonafir, 2017). Growth promoters are administered either as anabolic implants or dietary supplements (Davis and Blek, 2018). While dietary feed supplements such as probiotics are regarded as natural growth promoters, other non-nutrient additives are referred to as growth promoters or antibiotic growth promoters (AGP) (Vondrscova et al. 2010; Davis and Blek 2018; Hughes and Heritage 2004; Herago and Agonafir, 2017).

Antibiotics/antimicrobials administered as growth promotants are added to feed and function either as metabolic modifiers or impart prophylactic disease resistance. Antibiotic/antimicrobial growth promoters work by suppressing competitive populations of bacteria in the digestive tract to allow the animals to get the most nutrition from a diet by increasing nutrient availability (Hughes and Heritage 2004; Herago and Agonafir, 2017). Antimicrobial growth promoters have been suggested to strongly suppress the bacterial catabolism of urea and amino-acids, and decrease carbohydrates breakdown and bile salts decomposition. This leads to an increase in nutrient and energy levels available to the animal and decrease in the concentration of toxic byproducts like ammonia in the gut. In cattle, the rumen flora fermentation favours the production of propionic acid against acetic acid, allowing for increased deposit of muscle and decreased methane production (Corpet, 2000).

Growth promotors also include anabolic implants, which are inserted as pellets underneath the skin of the middle of the ear of the bovine. The hormones are released slowly over time, bypassing digestion. A combination of three naturally occurring hormones (estradiol, progesterone and testosterone) and 2 synthetic hormones (zeranol and trenbolone acetate) are anabolic agents used in beef cattle growth promoting implants (Al-Dobaib and Mousa, 2009).

The effects of growth promoters can be seen in a broad spectrum of growth performance measures of the animal, including feed conversion ratio, average daily gain, carcass quality, and palatability properties among others. Different studies have reported varied results on the effects of growth promoters on these production and quality measurements and these will be reviewed individually.

1.4.1 Effect on animal performance

Several studies have been performed on the effects of growth promoters on animal performance. Bhatt et al. (2016) reported improved digestibility, nutrient utilization and feed conversion ratio in rabbits receiving probiotic supplementation. In their report on the effects of repartitioning agents on animal performance, Avendano-Reyes et al. (2006) noted an improved ($P < 0.01$) gain to feed ratio and an increase in average daily gain (ADG) of 26% when cattle were treated with zilpaterol, and steers fed with ractopamine hydrochloride consumed less feed than control steers. The report of Jean et al. (2014) also supported the increase in the ADG of cattle fed with zilpaterol as well as a decrease in dry matter intake (DMI).

1.4.2 Effect on carcass traits

Ebarb et al. (2017) studied the effects of growth promoters on carcass quality and found an increase in the hot carcass weight and loin muscle areas of heifers treated with anabolic implants and ractopamine hydrochloride. There was no significant difference in carcass dressing percentage. Conversely, a meta-analysis done by Jean et al. (2014) reported a significant increase in dressing percentage as well as in hot carcass weight for cattle fed zilpaterol hydrochloride. Dressing percentage may or may not be affected by HGP, as it is heavily influenced by the animal weight at slaughter as dressing percentage is calculated as the hot carcass weight divided by the live animal weight multiplied by 100. Differences in the impact of steroids on dressing percentage may be due to differences in gut fill, as increased gut fill will decrease the dressing percentage by artificially increasing live animal weight.

1.4.3 Effect on meat quality

The use of BA such as ractopamine and zilpaterol hydrochloride has been shown to not affect beef palatability (Garmyn and Miller, 2014), flavour (Fernández-Dueñas et al. 2008), and toughness (Barham et al. 2013). Others, however, have claimed that use of growth promotants increased WBSF and subsequently decreased the tenderness of meat (Dikeman, 2003; Avendano-Reyes et al. 2006; Faucitano et al. 2008). The results in the literature are equivocal and therefore additional investigation into the effect of BA on beef tenderness is warranted.

1.4.4 Effect on the environment

The use of growth promoters may reduce greenhouse gases, volatile organic acids and ammonia emissions from feedlot cattle due to increased nitrogen retention (Stackhouse-Lawson et al.

2013; Ross et al. 2011) and decrease methane production (Dobaib and Mousa, 2009). This arguable benefit of using steroids has encouraged the persistence of this practice despite market pressures to suspend their use. For this reason, the use of steroids in cattle production will most likely continue in the Canadian beef industry, and so continued examination of their effect on beef eating quality is warranted.

1.5 Regulation of growth promoters

There has been a largely reluctant acceptance by the public of the use of growth promoters in beef production (Herago and Agonafir, 2017). This is due to concerns raised on the effect of growth promoter residues on human health or the development of antimicrobial resistant strains of human pathogens (Herago and Agonafir, 2017). These residues are argued to have adverse effects on human health and on the environment owing to their persistence in the environment, which ranges from weeks to months in manure and feed runoffs (American Public Health Association (APHA), 2009). The use of growth promoters in the beef industry follows strict regulations and different countries have policies regarding the use of growth promoters in animal production (Hughes and Heritage, 2014).

Sweden was the first country to ban the use of antibiotic growth promotants (AGP) in animal production because it might lead to development of antibiotic resistant organisms (Teillant, 2015). The European Union (EU) prohibited the use of any substance with hormonal, thyrostatic or beta-adrenergic action for use as growth promoters in food animals (Maron et al. 2013; Butaye et al. 2003; Hughes and Heritage, 2014). The implicated substances included progesterone, oestradiol 17ß, testosterone, zeranol, trenbolone acetate and melengestrol acetate (MGA) and all beta agonists. The advisory panel stated that there was no acceptable daily intake (ADI) that could be established for any of the hormones [European Union (EU) Commissions]. This prohibition was issued as Directive 96/22/EC, which was later amended into Directive 2003/74/EC. In addition to the EU ban on the use of growth promoters, countries such as Germany put additional policies in place to prohibit the use of antibiotics or antimicrobials as growth promoters, explicitly stating that they should be administered solely for the treatment of disease (GAIN Report, 2011).

There is no explicit ban on the use of growth promoters in Japan although a veterinary prescription is required for use of antimicrobials in animals. In Mexico, most AGPs have been

banned but exceptions are provided for fifteen drugs, including avoparcin, vancomycin, bacitracin, and tylosin (Maron et al. 2013; Teillant 2015). According to Maron et al (2013), there are no reports on the policies guiding the use of growth promoters in countries like Russia, Hong Kong, and the Philippines; however, Teillant (2015) stated that the use of AGP is allowed in non-OECD countries such as China, Brazil, Russia, Philippines, Argentina, US and Australia (Hughes and Heritage, 2014).

Growth promoters (hormones and beta agonists) approved for use in the US and other countries alike have been evaluated and approved by WHO/FAO (FAO, 2013). The US government posits that the use of growth promoters such as anabolic implants and their residual levels pose no threat to human health. Regulatory approval of the use of antibiotics for growth promotion in livestock has been based on demonstrable target animal safety, residual drug safety, edible tissue clearance and avoidance, and environmental safety, as well as measurable growth promoting effects (Jeong et al 2010; Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2000a). Despite this fact, in the US, several recommendations have been raised by different bodies to restrict or reduce the usage of AGP (Dibner and Richards, 2005), and more US public health organizations have taken formal stances to oppose the use of AGP (APHA, 2009).

In addressing these human health concerns, the Joint European Committee on Food Additives (JECFA) convened to evaluate the safety of these residues to human health. This committee was tasked with carrying out a risk assessment of these residues and to recommend limits to intake [acceptable daily intakes (ADI) and residue levels (maximum residue level (MRL)]. These limits were set after examining the toxicology, microbial risk and detectable residues (Jeong et al 2010). In analyzing toxicity, data pertinent to macromolecular binding, immune function, and short term and long-term carcinogenicity were utilized. For growth promoting compounds with antimicrobial activity, studies to evaluate the potential for adverse effects on the microbiological ecology of the human intestinal tract were also incorporated. In assessing microbial risk, the characteristics of the human gut flora were considered, as were indications of 'barrier effects' and factors determining bacterial growth. From these considerations, an estimate of the concentration without microbiological effect on the relevant microorganisms colonizing the distal part of the human intestine was made. This was based upon an estimate of the fraction of

the ingested amount of the antimicrobial substance that would be available to the bacteria in that part of the intestine.

With regard to residues, taken into consideration were the chemical identity and properties of the drug, its use and recommended doses, along with pharmacokinetic, metabolic and pharmacodynamic studies in experimental and food producing animals and humans where available. Also considered were residue depletion studies with radio-labelled drugs in target animals from zero withdrawal time to periods extending beyond the recommended withdrawal time. The latter studies would provide information on total residues, including free or bound residues and major residue components to permit selection of a marker residue and target tissue. Also considered were the routine analytical methods that may be used by regulatory authorities for the detection of residues in the target tissue.

1.6 Beta adrenergic agonists (BA)

Beta-adrenergic agonists are analogues of catecholamines, which are neurotransmitters naturally found in animals. Like epinephrine and norepinephrine, they are members of the phenethanolamine group of organic compounds, which bind to G-protein coupling receptors (Strydom et al. 2009; Tavares, 2011). β-Adrenergic agonists are widely used in cattle production to increase growth by maximizing lean tissue accretion, and these compounds repartition nutrients toward decreased lipogenesis, increased protein accretion, decreased protein degradation, or a combination of all these processes, (Wheeler and Koohmaraie, 1992; Byrem et al. 1998). Feeding of β-agonists may result in increased meat toughness due to reduced proteolysis post mortem (Geesink et al. 1993).

Beta-adrenergic agonists are an effective tool for enhancing lean tissue accretion and improve beef carcass cutability (Mersmann 1998; Beckett et al. 2009; Vogel et al. 2009). The mode of action of BA is well studied and complex. Johnson et al. (2014) reported that treatment of mammals with BA causes an increase in the amount of RNA transcript for several skeletal muscle proteins as well stimulation of adipocyte triacylglycerol degradation and inhibition of fatty acid and triacylglycerol synthesis. β-adrenergic agonists bind to certain beta receptors, altering biochemical processes of tissue growth by increasing lipolysis and decreasing lipogenesis (Mersmann 1998), stimulating hypertrophy of muscle fibers (Beermann et al. 1987), increasing synthesis of skeletal muscle protein (Johnson et al. 2014), reducing protein turnover

(Anderson et al. 1990), and reducing protein degradation (Johnson, 2014; Strydom et al. 2009; Wheeler and Koohmarie 1992). To avoid complications that may arise due to severe exposure, specifically 'rebound' at product use cessation which leads to an increase in fat deposition and a decrease in muscle, a BA is best administered one to two months before slaughter of the animal (Herago and Agonafir, 2017).

1.6.1 Ractopamine

Ractopamine hydrochloride (RAC) is the only BA currently marketed for use in cattle in North America (Allen, 2014). Ractopamine hydrochloride is a phenethanolamine that binds to the beta-adrenergic receptors (BARs) located on the surface of the cell, increasing synthesis of protein and reducing lipogenesis (Bryant et al. 2010; Quinn et al. 2016). Phenethanolamines have a substituted aromatic ring and an ethanolamine side chain with various substitutions on the aliphatic nitrogen (Tavares, 2011). As a B1-adrenergic agonist, it is fed as a finishing diet supplement to cattle before slaughter. Administration of ractopamine hydrochloride to cattle as part of a finishing diet leads to partitioning of dietary energy toward muscle protein accretion and increased myofibrillar total protein synthesis rather than adipose accretion (Mersmann, 1998; Anderson et al. 1990). Ractopamine hydrochloride is available for use in cattle as Optaflexx™ in Canada and as Actogain™ 45 in the US.

Substantial work has been done to characterize the effects of ractopamine hydrochloride on various properties of food animal production, including animal performance, carcass quality, meat palatability and effects on human health.

1.6.1.1 Effect of ractopamine on animal performance

Ractopamine hydrochloride increases the feed conversion ratio and average daily gain of cattle (Quinn et al. 2016; Boler et al. 2012; Strydom et al. 2009). Conversely, no effects on growth performance (Allen et al. 2009) or average daily gain and gain/feed ratio (Ross et al. 2011) have been noted. Avendano-Reyes et al. (2006) reported decreased feed intake ($P < 0.05$) and improved gain/feed ratio ($P < 0.01$) in steers. Treatment of pigs with ractopamine hydrochloride also resulted in increased ADG, average daily feed intake (ADFI) and gain to feed ratio (G:F) (Puls et al. 2015; Peterson et al. 2015). In lambs treated with ractopamine hydrochloride, it was observed that wool lambs fed 20 and 30 mg ractopamine hydrochloride had higher ($P<0.05$) total weight gain and lower feed conversion than

lambs fed 0 and 10 mg ractopamine hydrochloride. Because RAC has been extensively studied in pigs, cattle and lamb, the impact of RAC on domestic livestock growth is well understood.

1.6.1.2 Effect of ractopamine on carcass quality

Carcass quality is an important property that is commonly evaluated in studying the effects of ractopamine in animal production. While some studies have found that the use of ractopamine hydrochloride did not have any effect on carcass characteristics such as hot carcass weight (HCW), *longissimus* muscle area, or *longissimus* muscle area per 100 kg of HCW (Gonzalez et al. 2010; Romero- Maya et al. 2013; Quinn et al. 2016), others have reported an increase in live weight, HCW (Platter et al. 2008; Jean et al. 2014) and loin muscle area (Boler et al. 2012). Results have also shown an increase in dressing percentage and a decrease in the marbling score (Gonzalez et al. 2010; Romero-Maya et al. 2013; Quinn et al. 2016; Jean et al. 2014; Boler et al. 2012). Allen et al. (2009), however, reported an increase in marbling score. Clearly, further understanding of the effects of RAC on carcass quality is warranted given the inconsistency of carcass quality results related to this product.

1.6.1.3 Effect of ractopamine on consumer acceptance and sensory properties of meat

Most of the work studying the effects of ractopamine has shown changes in sensory properties where tenderness was the major focus (Platter et al. 2008). An increased dose of ractopamine hydrochloride led to an early post mortem increase in shear force value; however, no difference was observed between ractopamine hydrochloride-treated beef (200mg and 300mg) and the control samples after prolonged ageing (Boler et al. 2012; Ebarb et al. 2017). Weber et al. (2013) concluded that beef from cows fed with ractopamine hydrochloride had increased instrumental toughness values. Platter et al. (2008) reported that ractopamine hydrochloride increased the WBSF values of muscle from the carcasses of steers and heifers, with results similar to those of Arp et al. (2013) and Jean et al. (2014). Both Arp et al. (2013) and Jean et al. (2014) noted that ractopamine hydrochloride treatment had little impact on trained panel sensory ratings as there was no observable difference in beef flavour and juiciness. In studying the effect of treatment of pigs with ractopamine hydrochloride, Juarez et al. (2016) stated that all samples were acceptable to a consumer panel and that there were no observed differences on evaluated sensory properties of colour, flavour and odour. In contrast, Allen et al. (2009), from their study of the effects of ractopamine hydrochloride on beef from dairy cows, concluded that cows fed ractopamine had increased flavour intensity compared to those that did not receive it. Broadly speaking, based on the model developed by Platter et al. (2003), the changes in

WBSF due to ractopamine would result in a less than 4% shift in overall consumer satisfaction (Platter et al. 2008).

1.6.1.4 Effects of ractopamine on human health

The joint FAO/WHO Expert Committee on Food Additives (JECFA) confirmed the human safety standards for ractopamine at the 40th, 62nd and 66th meetings of the committee (JECFA FAO Monograph, 2010). After rigorous evaluations of risk assessments, the committee recommended an acceptable daily intake as well as a maximum residue level (MRL) for target organs of cattle and pig as shown in Table 1.1. Ractopamine administered to animals at approved doses coupled with adherence to recommended withdrawal times have shown little to no level of residues in humans (Johnson, 2014).

Table 1.1: Comparison of approved MRLs by Codex Alimentarius Commission (CAC), USA and Canada (Source: CAC/MRL 2-2015; Health Canada)

Species	Tissue	MRL (ppm)		
		CAC	USA	CANADA
Cattle	Muscle	0.01	0.03	0.01
Cattle	Liver	0.04	0.09	0.04
Cattle	Kidney	0.09	-	0.1
Cattle	Fat	0.01	-	-
Pig	Muscle	0.01	0.05	0.04
Pig	Liver	0.04	0.15	0.12
Pig	Kidney	0.09	-	0.14
Pig	Fat	0.01	-	-

1.7 Hormones

Hormones are a group of signaling molecules of the endocrine system in multicellular organisms that are transported via the circulatory system to target distant organs for physiological and behavioral regulation. They are of diverse structures but can be broadly categorized as eicosanoids, steroids or amino acid derivatives. Hormones act as growth factors, altering growth rate or body composition. The role of hormones in homeostatic mechanisms include energy regulation, mineral or water balance, and lipid, protein and carbohydrate metabolism (Al-Dobaib and Mousa, 2009). Steroid hormones are produced from cholesterol and secreted by the steroid glands. Naturally produced steroid hormones are

classified according to the organ that synthesizes them; specifically, adrenal hormones and the gonadal hormones (https://www.britannica.com/science/steroid-hormone).

Gonadal hormones have two major functions in the body; androgenic and anabolic effects. The androgenic functions include growth of penis (male) or clitoris (female), and growth and development of seminal vesicles and prostate glands (male). On the other hand, the anabolic functions include promoting muscle growth, increasing muscle mass, enhancing immune system function, and nitrogen retention. Synthesized androgenic steroids are hormones that are manufactured to favorably induce the anabolic effects of these hormones while suppressing the androgenic effects (https://www.differencebetween.com/difference-between-anabolic-and-androgenic/). In total, six hormones are permitted for use in beef production in Canada: the three naturally occurring gonadal hormones (testosterone, progesterone and estrogen) as well as their synthetic counterparts (the estrogenic compound zeranol, the androgen trenbolone acetate, and the progestin melengestrol acetate) (OCA, 2007; Al- Dobaib and Mousa, 2009). These hormones have been reported to improve carcass quality by reduction of fat, increased lean meat production and increased feeding efficiency. These are associated with reduced cost of production and increased profits (Al- Dobaib and Mousa, 2009; Dikeman, 2007). Anabolic steroid hormones have been reported to improve muscle growth by competing with glucocorticoids for receptor sites on muscle cell membrane. Glucocorticoids have catabolic effects on tissue; therefore, their displacement would reduce muscle cell catabolism and muscle protein degradation (Velle, 1981; Preston, 1999). Estrogenic hormones increase circulating levels of insulin-like growth factors (IGF-1) and somatropin (Velloso, 2008). Steroid hormones are implanted into cattle at various stages of growth (Al- Dobaib and Mousa, 2009).

1.7.1 Hormonal implants in beef production

Implants are small pellets encapsulating the hormones and they slowly release the hormones over time (Stewart, 2013). Apart from MGA, which is a progestin that is given orally as a feed supplement to intact and open (not pregnant) slaughter heifers, the only approved application for hormones is implantation. The implants are subcutaneous, typically placed under the skin at the back of the animal's ear as the ear is generally not used for human food and is discarded at slaughter, thus reducing food supply contamination (Galbraith, 2002; Herago and Agonafir, 2017; Velle, 1981; US FDA, 2017). Factors to be considered when implanting cattle are: animal age (calves, feeders or finishing cattle); sex (male or female); breed (exotic or British); nutritional program; and carcass considerations (age, breed, and sex) (Lehmkuler and Burris, 2010).

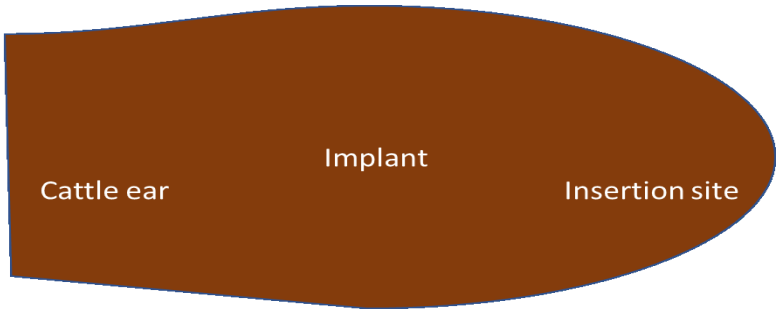

Figure 2: Location for placing implants in cattle ears

1.7.2 Effects of hormonal implants on beef quality

Hormones have been used for over 50 years in beef cattle production either singularly or in combination. Hormonal implants have more pronounced effects on steers and heifers than bulls and an increase in fat deposition has been reported for bulls with implants (Dikeman 2007, Patterson and Salter, 1985). Estrogenic and androgenic implants were more effective in steers and heifers respectively and combination implants, implants that have both estrogenic and androgenic compounds, have an amplifying effect in both steers and heifers (Dikeman 2007). Several reports have been given on their effects and there is consensus on the effects of hormonal implants on carcass quality and feed conversion efficiency. Hormonal growth promotants appear to produce an increase in growth rate (10% to 30%); in feed conversion efficiency (15% to 25%), and an increase in carcass leanness (5% to 8%) (Dikeman 2007; Hutcheson, 2008; Preston, 1999; Hutcheson et al. 1993; Lopez-Campos et al. 2012; Trenkle and Burrow, 1978; Heitzman, 1979). Hutcheson et al (1993) reported increased average daily gain on re-implantation. This is also supported by the review of Preston (1993), and this lends credence to the observation that the impact of hormonal implants peaks and subsequently declines. Re-implantation therefore helps to ensure a steady advantage in growth and feed efficiency.

A review by Dikeman (2003) concluded that implanting cattle reduced the tenderness of beef and was associated with decreased marbling. Lean et al. (2018) had similar findings, and they reported an increase in the WBSF values of beef from cattle with hormonal implants, further concluding that ageing did not significantly change the WBSF values. Lopez-Campos et al. (2012) observed a significant decrease in marbling score with the use of hormonal implants but there were no significant differences in marbling scores between calf-fed and yearling-fed steers. This contrasts with Brewer et al (2007) who reported lower marbling scores for yearling-fed compared to calf-fed cattle. Platter et al (2003) studied the effect of implanting cattle at different phases of production (branding, weaning,

backgrounding, feedlot entry or re-implantation). He reported that implanted steers regardless of production phase had lower marbling scores than the control (non-implanted steers), and that production phase had no effect on marbling scores. Additionally, in that study, marbling scores was reduced as the number of implanting times increased. Schneider et al. (2007) reported that implanting cattle once did not affect marbling score, the incidence of a carcass grading USDA Choice or WBSF value but found that re-implanting led to a decrease in these measurements. Other authors observed no difference in marbling scores between cattle implanted and those not implanted, thereby reporting little or no effects of hormonal implants on marbling score (Hutcheson, 2008; Duckett et al. 1996). The disparities in the results of the various studies reviewed may have arisen due to the use of different breeds, ages, and genders of cattle, as well as differences in the timing and aggressiveness of the implants studied (Garymyn and Miller, 2014; Nicholos et al. 2002).

A consumer study carried out by Roeber et al. (2000) showed reduced consumer preference for steaks from implanted cattle. Nichols et al. (2002) reviewed 19 studies investigating the effects of hormones on beef toughness, of which only 3 noted an increase in the WBSF values with implanted cattle. Interestingly, 2 of the reports showed decreased WBSF with implanted cattle. These results suggested that it is difficult to make a definite conclusion as to the effect of hormonal implants on palatability properties of beef especially regarding beef toughness. Barham et al (2003) reported lower WBSF values for implanted beef at 3 and 7 days post mortem ageing but no difference in values between non-implanted and implanted beef after 21 days of post mortem ageing, concluding that moderate levels of implants had no detrimental effect on beef tenderness. found the opposite, with WBSF increased in cattle that received. The results of Barham et al. (2003) indicate that the effect of implants on beef WBSF may vary with days of aging, with the impact of steroid use most likely evident early post mortem. Given that the effect of aging may be greater than that of the steroid treatment itself, co-varying for days of aging when comparing studies on the effects of steroid implants on meat toughness is warranted.

1.7.3 Government regulation of hormonal implants in beef production

The use of hormonal implants in beef production is prohibited in Europe. The major concern is the adverse effects of intramuscular hormone residues on consumer health (Galbraith, 2002). It is argued that although some of the hormones used in implants are naturally occurring, their

concentration varies with sex, age and physiological state and their use in beef production exposes individuals to higher concentrations than naturally expressed. An instance of this is that the natural hormone 17β-oestradiol which has been linked to genotoxic potential (Herago and Agonafir, 2017). Regulating bodies such as the US Food and Drug Administration (FDA), Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have concluded that such levels of hormone implants were too minimal to pose a threat to humans. It was concluded that hormones used in compliance with good agricultural practice and recommended levels pose no threat to human safety as there is no possibility that residues will exceed set tolerance limits (Preston, 1999). Also, there is presently no data showing evidence of toxicity potential at levels below those stipulated (Herago and Agonafir, 2017) and approved for use in beef production. The regulatory levels for use of hormonal implants according to the Joint FAO/WHO JECFA are given in Table 1.2. The US FDA (2017) claims that all approved steroid implant products have a zero-day withdrawal, implying their safety for consumption at any time after the animal has been treated. Recent results of risk assessments on natural steroid hormones (estradiol-17β, progesterone, testosterone) showed that they have little or no impact on humans when used as recommended and as such do not have average daily intakes (ADIs) or maximum residue limits (MRLs) as they are already produced in varying concentration within the body. However, for synthetic hormones (zeranol, trenbolone, and melengestrol acetate) ADIs and MRLs are specified for human safety (JECFA, 2000).

1.8 Residual Feed Intake (RFI)

Recently, because of market restrictions increasingly put into place by countries such as Europe and China due to concerns with the safety of product from cattle treated with steroidal hormones and beta-adrenergic agonists, increasing cattle feed efficiency through genetic selection has received significant attention. Residual feed intake is defined as actual feed intake minus the expected feed intake of each animal. Koch et al. (1963) was the first to propose RFI as an alternate measure of feed efficiency. Koch et al. (1963) suggested that feed intake could be adjusted for body weight and weight gain, effectively partitioning feed intake into two major components: the feed intake that is expected for a given level of production, and a residual portion. The residual portion of feed intake can be used to identify animals that deviate from their expected feed intake. There are usually two types of RFI category animals: high and low.

Table 1.2: Toxicological endpoints and regulatory limits of hormonal growth promoters (source: JECFA, 2000)

Compound	Toxicological endpoint	NOAEL (μg/kg BW/day)[1]	ADI[2](μg/kg BW/day)	MRLs[3] (μg/kg) for cattle tissues
17β-estradiol	Relief of the symptoms of menopause and changes in the serum concentrations of corticosteroid-binding globulin	5	0~0.05	Unnecessary
Testosterone	Androgenic effects	1,700	0~2	Unnecessary
Progesterone	Changes in the human uterus	3,300 (LOAEL[4])	0~30	Unnecessary
Zeranol	Estrogenic effects	50	0~0.5	2 (muscle), 10 (liver)
Melengestrol acetate	Changed menstrual cycle	5	0~0.03	1 (muscle), 10 (liver) 2 (kidney), 18 (fat)
Trenbolone acetate	Androgenic effects	2	0~0.02	2 (muscle, β-trenbolone) 10 (liver, α-trenbolone)

[1]No observed adverse effect level (NOAEL) μg/kg human body weight/day.

[2] Acceptable daily intake (ADI) μg/kg human body weight/day.

[3] Maximum residual levels (MRLs).

[4] Lowest observed adverse effect level (LOAEL).

Low RFI animals consume less feed than but attain a similar body weight to high RFI animals. Herd et al. (2003) concluded that selection for low post-weaning RFI in heifers can result in reduced feed intake and an increase in feed efficiency of the breeding herd. This means that selection for low RFI in growing animals will achieve lower RFI in breeding females, which ultimately will reduce the feeding cost of the cow herd. RFI is said to be the best measure of feed efficiency because it is not dependent on the level of production or performance; also, RFI is heritable (h^2 = 0.16 to 0.43) (Herd et al. 2003), and so it responds to genetic selection (Sainz and Paulino 2004).

1.9 Sensory Analysis

A common method of evaluating the beef palatability properties is sensory evaluation. "Sensory evaluation has been defined as a scientific discipline used to evoke, measure, analyze and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing" (Stone and Sidel 1993). As a scientific method of analysis, sensory evaluation should be carried out in controlled conditions with appropriate experimental designs, test methods and statistical analyses to ensure validity and proper interpretation of obtained results (Singh-Ackbarali and Maharaj, 2014).

Sensory analysis can be broadly classified as product-oriented or consumer-oriented (Adjei, 2017). These classifications are based on the aim of the analysis, which is either to understand a product or to gauge consumer perception of a product (Caliman, 2016). To understand a product, panelists are usually trained to be attuned to specific product attributes or characteristics, while consumer panelists are usually asked to describe their perception of product attributes or acceptability (Heymann and Ebeler, 2017). Trained panelists, therefore, are used to conduct an analytical sensory evaluation, while an untrained panel is used to conduct consumer or hedonic sensory evaluation (Lawless and Heymann, 2010). In analytical sensory evaluation, the focus is on the characteristics of the product being tested, and the data collected are considered objective because panelists are used as instruments. Trained panels are the gold standard for objective description of product attributes because no machine can replicate the complex interactions that occur in an individual's sense organs when a food product is ingested. In consumer sensory evaluation, the data collected are considered subjective because the data represent the consumer's raw response to the product (Heymann and Ebeler, 2017). Because panelists in an analytical panel are trained or "calibrated", data collected from trained panelists

does not represent consumer opinion. The development of each type of panel differs as a result of their different objectives.

1.9.1 Trained Descriptive panel

This is a sophisticated sensory test that gives quantitative values to all sensations perceived (Stone et al. 2012; Singh-Ackbarali and Maharaj, 2014). Properties analyzed include aroma, flavour, texture, and sounds that distinguish a product from other products. Several descriptive tests methods have been developed and tailored to suit different sensory philosophies (Murray et al. 2001). Generally, descriptive testing involves screening of many candidates for their ability to sense and describe specific well-known aromas, flavours, textures or sounds and those that perform well are selected for the further training. The selected panelists are then trained to understand the attributes to be evaluated and to create a common sensory language among the panelists to use to describe the attributes. This can be built by the panelists or can already be an existing sensory lexicon that is adopted by the panelists. Subsequently, a frame of reference for each attribute is introduced and used to anchor the perception of the panelists to a common level of understanding to minimize variation between panelists so that differences between products can be detected. This serves as a focal point to which panelists refer while evaluating products. Following training, the product is evaluated, the data are collected, and statistical analysis of the data ensues (Murray et al. 2001; Singh-Ackbarali and Maharaj, 2014; Heymann and Ebeler, 2017).

1.9.2 Consumer panel

These panels are employed to evaluate the level of consumer acceptance of a product. They are performed using an untrained panel, which usually means that there is substantial variation between panelists in the use of the sensory assessment tool. To detect differences between products then many participants are needed and consumer panels with more than 60 panelists are common (MacFie et al. 1989). Methods of consumer preference testing include paired preference techniques, hedonic ranking, hedonic scaling, and conjoint analysis (Heymann and Ebeler, 2017; Singh-Ackbarali and Maharaj, 2014).

1.10 Sensory evaluation of meat

Both consumer and trained panels have been used to evaluate the sensory properties of meat (Lorenzen et al. 2003; Bruce et al. 2005; Lucherk et al. 2016; McKillip et al. 2017; O'Quinn et al. 2018). The American Meat Science Association provides guidelines for discrimination,

descriptive and consumer sensory evaluation of meat (AMSA 2016). The guidelines indicate that discrimination testing, such as triangle, duo-trio, or degree of difference from control tests, is used to determine if a difference between two products is to be detected, and this type of test can be used with either trained or consumer panels, although trained panels are likely to be more sensitive to differences than consumers (AMSA 2016). Descriptive analysis methods encompass flavour and texture profiling and descriptive attribute analysis, evaluation and magnitude estimation (AMSA 2016). Although descriptive panels are usually trained, Generalized Procrustes Analysis has been used to reduce variation between untrained panelists during free choice profiling of beef (Bruce et al. 2005).

Trained panels are favoured when sample volume is limited because consumer studies can demand large volumes of meat, and thus large numbers of animals. The limitation with this type of panel is that the acceptability of the product cannot be measured, although research has evaluated the alignment of trained panels with consumer panels and found that correlation of between 0.67 and 0.70 and 0.62 and 0.75 for beef tenderness and juiciness, respectively (Lucherk et al. 2016; McKillip et al. 2017). O'Quinn et al. (2018) found that meat tenderness, flavour and juiciness accounted for 43, 49 and 7% of the variability in overall palatability. O'Quinn et al. (2018) also found that if meat tenderness, juiciness, or flavour was deemed unacceptable by a consumer, the likelihood of a steak being rated unacceptable overall was 69, 66 and 77%, respectively, indicating that juiciness is a very important indicator of overall satisfaction with beef. In light of this, consideration of sensory tenderness, juiciness and flavour is important for trained meat sensory panels as well.

1.11 Hypotheses and objectives

As indicated in the preceding review, gaps exist in our understanding of the effects of hormonal growth promotants and residual feed intake on the palatability of beef, particularly of high connective tissue muscles. I hypothesize that steroids will increase the toughness and reduce the sensory tenderness of beef and interact with selection for low residual feed intake to additionally toughen beef. I also hypothesize that supplementation of beef steers during the finishing period with ractopamine will increase sensory tenderness rating of beef regardless of growth promotant use. Therefore, the objective of this thesis is to identify the effects of genetic selection for residual feed intake and its interactions with growth promotant use and ractopamine

hydrochloride on the sensory characteristics of beef from one low and two high connective tissue muscles.

CHAPTER 2: Materials and Methods

Beef production was performed following the guidelines of the Canadian Council on Animal Care (1993) and was approved by a research animal ethics committee at the University of Alberta (AUP00001801). Approval for the use of human subjects in the trained panels was received from a Research Ethics Board at the University of Alberta following review of the study protocol for its adherence to ethical guidelines (PRO00073730). All participants provided written informed consent prior to participation in the study and all participants received an incentive in the form of gift cards at the completion of each sensory panel.

2.1 Experimental design and animal and sample management

Forty-eight (n=48) Angus crossbred bull calves were born from April to June, 2015, at the University of Alberta Kinsella ranch and each calf was identified by a unique ear tag. Calves were castrated within 8 weeks of birth by elastration and remained with their dams on pasture at the ranch until weaned. At weaning, steers were weighed and then gradually put on a primarily forage background diet (barley silage 72%, oats 21%, canola meal 4% and Rumensin^{TM}/mineral premix 3%) for approximately 5 months. Steers were sorted by RFI status (low n = 27, high n = 28), stratified by weaning weight and then randomly assigned to receive steroids (n = 24) or not (n = 24), and ractopamine (n = 23) or not (n= 25) 2 x 2 x 2 factorial design (Figure 2.1).

As steer body weight and feed intake increased, the steers were graduated onto a finishing diet of barley silage (27%), barley grain (61%), and canola meal (8%), with a Rumensin™ and mineral supplement (4%). A mineral and protein supplement (Feedlot 32, Cargill, Camrose) was included for the last 120 days of finishing. The steers were implanted twice, the first implantation at about 320 days of age with a progesterone and estradiol-based implant (200 mg progesterone, 20 mg estradiol benzoate and 29 mg tylosin tartrate) (Component E-S, Elanco Animal Health, Greenfield, Indiana, USA), with a second implant (120 mg trenbolone acetate and 24 mg estradiol) (Component TE-S, Elanco Animal Health, Greenfield, Indiana, USA) administered 80 days after the first diet until they reached a minimum 2 mm back fat at the 12th - 13th rib site, the requirement for a carcass to be eligible for Canada A quality grades. Ractopamine hydrochloride was fed to the cattle for the last 28 days prior to slaughter (200 mg/head/day) and administered with the mineral and protein supplement. Eight steers were slaughtered in a randomized complete block design over a period of 6 weeks at a provincially-

inspected abattoir in Vegreville, Alberta. Carcasses were chilled within an hour of exsanguination. At 48 h post mortem, the target muscles (*m. longissimus thoracis, m. gluteus medius* and *m. semimembranous*) were removed whole from the right sides of the carcasses. At 72 h post mortem, muscles were fabricated into 2.5 cm thick steaks. For sensory analysis, two steaks from each carcass were packaged individually under vacuum, with one not aged further (3 days aged) while the other was aged at 4 ± 1°C to 12 days post mortem. After ageing, all steaks were frozen at -20 ± 1°C at Agri- Food Discovery Place (AFDP), University of Alberta, and moved to the University of Alberta Food Laboratory when it was time for evaluation.

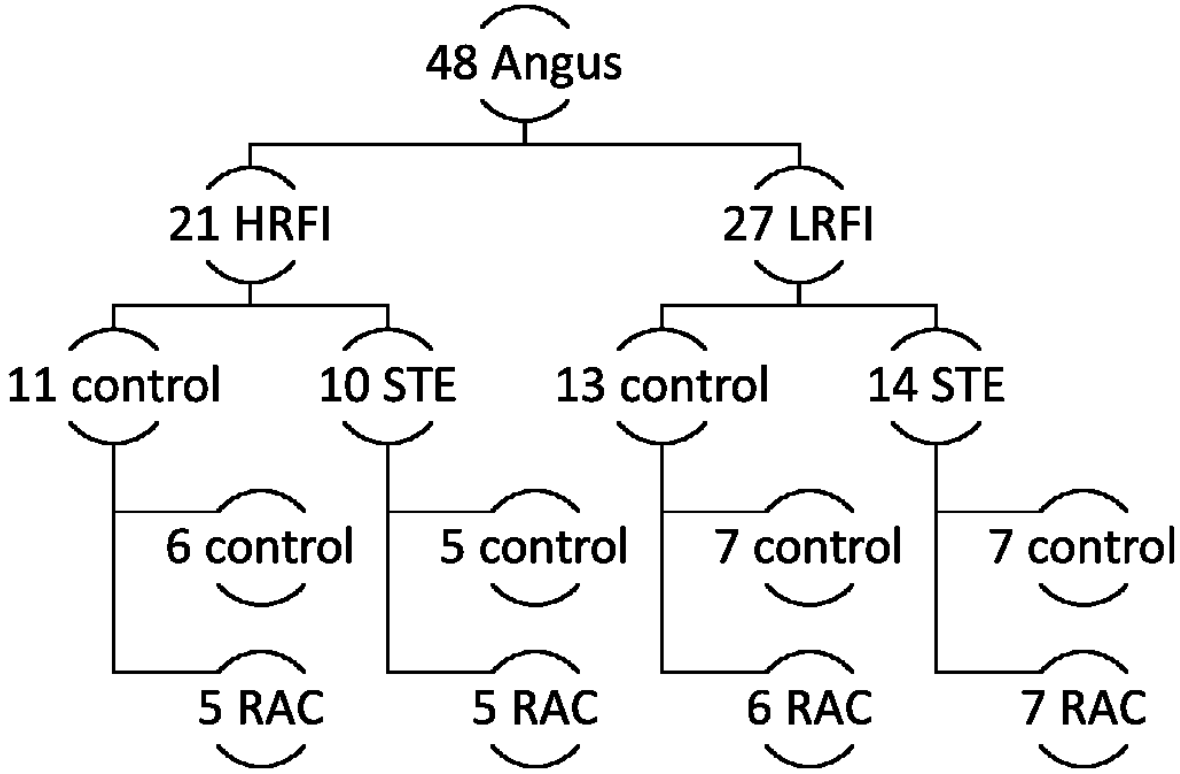

Figure 2.1: Experimental design. HRFI (high residual feed intake), LRFI (low residual feed intake), STE (steroid), RAC (ractopamine).

2.2 Experimental design for sensory evaluation

Each treatment combination was assigned numbers from 1-8 for the purpose of randomization (Table 2.1). Randomization was performed using the Compusense ® Cloud software (Compusense Inc., Guelph, Ontario, Canada). A Williams Latin Square design was used to achieve a balanced design and to avoid carry-over and tasting position effects (MacFie et al. 1989). Treatments were assigned at random within rows and columns of the Latin square, with each treatment appearing once per row and once per column. Animal production treatments were randomized across 12 sessions, with four treatments presented at each session. Within each session, beef aged 3 and 12 days from each animal was presented, so that the effect of ageing on beef sensory attributes could be assessed.

Table 2.2: Numbers allocated to each experimental treatment or sensory analysis within each ageing treatment

Residual feed intake	**Steroid**	**Ractopamine**	**Treatment number assigned for the Latin square**
Efficient	Yes	Yes	1
Efficient	No	No	2
Control	No	No	3
Efficient	Yes	No	4
Efficient	No	Yes	5
Control	Yes	No	6
Control	No	Yes	7
Control	Yes	Yes	8

2.3 Sample preparation

Steaks were removed from the freezer and thawed at 4°C in their packages for approximately 24 h at the University of Alberta Food Laboratory before evaluation. Before cooking, the steaks were allowed to rest at room temperature for about 20 min. The weights of the packaged steaks, their initial raw weights and that of the cooked steaks were recorded and used to determine purge and cooking losses. Samples to be grilled on a clam shell grill were removed from the vacuum pack and dried with clean paper towel to remove surface moisture and purge. Cuisinart multi-

functional product clam shell grills (Model CGR-4NC, 100 Conair Parkway, Woodbridge, Ontario, Canada) were used to cook the steaks according to American Meat Science Association Guidelines (2015) with some modifications. A thermocouple (DOT Black TX-1200-BK, DOT™ ThermoWorks, Inc. Utah, USA) was placed into the geometric center of each steak to monitor the internal temperature during cooking until a final temperature of 71°C was attained. Cooked steaks were then trimmed of visible fat and connective tissue and cut into cubes of approximate 2.54 cm thick ×1.27 cm wide × 1.27 cm long, and two cubes were then placed in aluminium foil and placed in a covered casserole dish in a 60ºC water bath to keep the meat samples warm prior to serving. The meat samples were then put into a 3-digit code labeled foam container with a plastic lid, and then served to each panelist. Panelists were provided with tepid water and unsalted crackers to cleanse their palates between samples. The samples were evaluated under white light to represent daylight by panelists who were situated in individual sensory booths.

2.4 Trained sensory evaluation

A trained panel was used to objectively determine the aroma, flavour and texture characteristics of each of the three muscles, with each muscle assessed by a trained panel individually, to determine differences in these characteristics due to selection of cattle for RFI, steroid implantation and ractopamine supplementation. Potential panelists were recruited from the University of Alberta North Campus (Appendixes A and B) and informed consent was obtained (Appendix C). Panelist candidates were screened using a questionnaire (Appendix D), in which each was asked to indicate their availability, to indicate any health conditions they may have had, to confirm that they consumed beef at least once bi-weekly, that they were able to describe sensory properties of common foods, and to ensure that they did not meet the exclusion criteria for the experiment (Appendix D). Panelists (n=8) were selected based on their screening results and their ability to attend all training sessions and all 12 sensory evaluation sessions. Panelists were trained in accordance with the American Meat Science Association (AMSA) sensory guidelines (AMSA, 2016) and a lexicon was developed for the attributes, with physical reference standards and their respective locations on the scale determined as well (Appendix E). Initial tenderness was defined as the initial force required to bite through the grain of a sample using the front teeth, with samples rated from 1, which indicated not tender, to 15, which indicated very tender. A score of 10 was considered tender and was equivalent to the force required to bite through the centre of a commercial meat ball. Overall tenderness was defined as the total force

used to chew a sample multiple times before swallowing, with samples rated from 1, which indicated not tender, to 15, which indicated very tender. A score of 12 was considered tender and was equivalent to the force required to chew a commercial meat ball multiple times. Juiciness was defined as the amount of perceived juiciness/moisture during chewing, with samples rated from 1, which indicated not juicy, to 15, which indicated very juicy. A score of 8 was considered normal juiciness for beef steak. Beef flavour intensity was defined as the intensity of the aroma and flavour generally associated with beef flavour in the sample, with samples rated from 1, which indicated weak beef flavour, to 15, which indicated strong beef flavour. Beef broth was considered a score of 5, ground beef was considered a score of 7, beef steak was considered a score of 8, and spiced ground beef was considered a score of 11. Brown/roasted was defined as the intensity of the aroma and flavour generally associated with beef that had been broiled, with samples rated from 1, which indicated weak brown/roasted, to 15, which indicated strong brown/roasted. For brown/roasted, ground beef and beef steak were considered to have scores of 8. Bloody/serumy was defined as the aroma and flavour generally associated with blood on undercooked or rare meat, with a score of 1 being weak bloody/serumy and 15 being strong bloody/serumy. For bloody/serumy, ground beef was considered a score of 2. Fat-like was defined as the aroma and flavour generally associated with cooked animal fat, with a score of 1 indicating weak fat-like and of 15 indicating strong fat-like aroma and flavour. Liver-like was defined as the aroma and flavour associated with cooked meat liver, with scores of 1 and 15 indicating weak and strong liver-like aroma and flavour, respectively, with beef liver having scores from 12 to 14. Sulphur-like/off-flavours were defined as the aroma and flavour generally associated with sulphur-like/off-flavour of meat, with scores of 1 and 15 indicating weak and strong aroma and flavour, respectively, with ground beef having a score of 1. After-taste was defined as the strength of the beef taste in the mouth after swallowing, with 1 and 15 indicating weak and strong after-taste, respectively, and beef steak being a score of 5.

Panelist performance was gauged during training with a three day assessment period. For three consecutive days, panelists were served the muscle of interest over 3 sessions (1 session/day) to gauge the repeatability and reproducibility of the training received. For sensory evaluation of experimental samples an 8 x 8 Williams Latin square design was used to avoid both presentation position and carry over effects (AMSA, 2016). Each panelist tasted 8 treatments in each of 12 sessions and each steak sample was evaluated by the eight panelists in each session. Preparation

for and execution of the panels followed the same procedure for each of the sensory sessions and for all the muscles used (Appendix G). The muscles were evaluated in 3 separate panels, with one panel for the LT muscle, a second panel for the GM muscle, and the third panel for the SM muscle. References standards were prepared and served at the same time as experimental samples. A 2.5 cm beef steak from the same type of muscle being evaluated was prepared identically to the experimentally samples, while commercial meatballs (frozen sirloin beef meatballs, M&M Food Market) were heated in an oven at 205°C for 20 min on a foil-lined tray. Following cooking, meatballs remained whole while the beef steak was cubed similarly to that of experimental samples. Both were wrapped in foil and warmed at 60 °C in a glass-lidded ceramic container until sensory evaluation, and panelists sampled the reference samples just prior to evaluation of the experimental samples.

The sensory questionnaire was presented using Compusense® Cloud software (Compusense, Guelph, Ontario) on tablets. In the questionnaire, panelists rated each sample for the following attributes: “Initial tenderness (IT)”; “Overall tenderness (OT)”; ”Juiciness”; “Beef flavour identity (BFI)”; “Brown/roasted (BR)”; “Bloody serumy (BS)”; “Fat-like flavour (FL)”; “Liver-like (LL)”; “Sulphur like/off-flavour (SL)”; and ”Aftertaste (AT)” on a 15-point category scale (15 = very tender, very juicy, strong beef flavour, strong brown roasted, strong bloody/serumy, strong fat-like, strong liver-like, strong sulphur/off flavour, strong aftertaste and 1 = not tender, not juicy, weak beef flavour, weak brown roasted, weak bloody/serumy, weak fat-like, weak liver-like, weak sulphur/off flavour, weak aftertaste respectively) (AMSA, 2016). Panelists rated the attributes using a questionnaire with the reference standard clearly marked (Appendix F).

2.5 Statistical analysis

Descriptive statistical analysis was performed using R statistical software version 3.3.1 (RStudio Team (2015), Boston, MA URL http://www.rstudio.com/). Sensory data were analyzed within muscle using a split plot design, with residual feed intake, steroid status and ractopamine status analyzed as a 2 x 2 x 2 factorial in the whole plot, and post mortem ageing time as the fixed effect in the sub-plot interacting with the fixed factors of the whole plot. The experimental unit for the whole plot was animal, and that of the sub-plot was steak. Where sources of variation in the model were significant at $P < 0.05$, differences between least square treatment means were determined using the Tukey’s Honestly Significant Difference test with significance at $P < 0.05$.

CHAPTER 3: Results

Sensory attributes of LT, GM and SM muscles from low and high (control) RFI Angus crossbred steers treated with either steroids and or RAC, or both or neither, were evaluated by 8 trained sensory panelists. Sensory data were analyzed within muscle; therefore, the results are presented by muscle.

3.1 M. longissimus thoracis

For overall tenderness, significant interactions existed between steroid use and post mortem ageing (P = 0.02) (Figure 3.1), steroid use and residual feed intake (P = 0.02) (Figure 3.2), and ractopamine use and post mortem ageing (P = 0.039) (Figure 3.3). Mean overall tenderness score of the LT was decreased by steroid use at day 3 post mortem, but there was no difference between LT steaks from carcasses of cattle treated with steroids and those from carcasses of control steers by day 12 post mortem (Figure 3.1), indicating that ageing alleviated this initial toughness. Also, the mean overall tenderness score of LT from high RFI steers was reduced when the steers were treated with steroids, but there was no effect of steroid treatment in the steers that were selected for low RFI (Figure 3.2).

The interaction between ractopamine status and post mortem ageing indicated that the mean overall tenderness scores for the LT was not different due to ractopamine status, but did differ between post mortem ageing times (Figure 3.3). This interaction was driven by a differential response to ageing of LT from carcasses of control steers and those that were fed ractopamine, with those fed no ractopamine having a greater increase in their mean overall tenderness score with ageing, although the lack of differences between the means did not reflect this.

For juiciness, there was an interaction between residual feed intake and steroid use (P = 0.0007), with LT from carcasses of high RFI steers that did not receive steroids having the highest score for juiciness, similar to that from carcasses of low RFI steers that received steroids, but higher than that of high RFI steers that received steroids and low RFI steers that did not receive steroids (Figure 3.4). Juiciness was not affected by any other interactions, nor was it affected by ractopamine use (Tables 3.1, 3.2 and 3.3). Mean juiciness score increased with post mortem ageing (P = 0.025) (Table 3.4).

Residual feed intake and steroid treatment interacted (P = 0.0289) for the attribute “bloody/serumy”, with mean panelist scores for this attribute greater in high RFI steers that did

not receive steroids than in low RFI steers that did not receive steroids (Figure 3.5). There were no effects of ractopamine or post mortem ageing on the perception of bloody/serumy flavour by panelists, nor any other significant interactions. Liver-like flavour was increased in LT from carcasses of high RFI (control) cattle (P = 0.018) (Table 3.3), but unaffected by all other treatments (Tables 3.1, 3.2, and 3.4) or interactions. Sulphur-like flavour increased with post mortem ageing (P = 0.043) (Table 3.4), but was unaffected by other treatments and interactions.

Table 3.1: Effects of steroids on the least squares means (and standard errors) of sensory attributes of the m. *longissimus thoracis*

Sensory attributes[1]	Steroid		P value[2]
	Treated	Control	
n	23	24	
Overall Tenderness	9.27(0.26)	10.37(0.25)	0.005
Juiciness	7.55(0.22)	8.17(0.22)	0.107
Beef Flavour Identity	8.83(0.11)	8.90(0.1)	0.751
Brown/Roasted	8.01(0.21)	8.14(0.21)	0.571
Bloody/Serumy	1.55(0.1)	1.42(0.1)	0.254
Fat-like flavour	0.76(0.06)	0.76(0.06)	0.990
Liver-like flavour	0.16(0.02)	0.19(0.02)	0.281
Sulphur-like flavour	0.03(0.01)	0.04(0.01)	0.427
Aftertaste	4.02(0.16)	4.12(0.15)	0.632

[1]Sensory attributes were evaluated on a 15-point descriptive scale.

[2]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.05$.

Mean panelist scores for LT beef flavour intensity, brown/roasted flavour, fat-like flavour and aftertaste were unaffected by treatment of cattle with steroids (Table 3.1), ractopamine (Table 3.2), selection for residual feed intake (Table 3.3) or post mortem ageing (Table 3.4).

Table 3.2: Effects of ractopamine on the least squares means (and standard errors) of sensory attributes of the m. *longissimus thoracis*

Sensory attributes[1]	Ractopamine		P value[2]
	Treated	Control	
n	22	25	
Overall Tenderness	10.05(0.26)	9.60(0.25)	0.240
Juiciness	8.07(0.22)	7.65(0.22)	0.180
Beef Flavour Identity	8.95(011)	8.79(0.10)	0.331
Brown/Roasted	7.9(0.21)	8.25(0.21)	0.316
Bloody/Serumy	1.5(0.1)	1.47(0.1)	0.778
Fat-like flavour	0.81(0.06)	0.7(0.06)	0.255
Liver-like flavour	0.18(0.02)	0.17(0.02)	0.973
Sulphur-like flavour	0.04(0.01)	0.02(0.01)	0.206
Aftertaste	4.07(0.16)	4.08(0.15)	0.972

[1]Sensory attributes were evaluated on a 15-point descriptive scale.

[2]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.05$.

Table 3.3: Effects of RFI on the least squares means (and standard errors) of sensory attributes of the m. *longissimus thoracis*

Sensory attributes[1]	Residual Feed Intake		P value[2]
	Efficient	Control	
n	26	21	
Overall Tenderness	9.79(0.24)	9.86(0.27)	0.814
Juiciness	7.7(0.21)	8.02(0.23)	0.266
Beef Flavour Identity	8.9(0.1)	8.83(0.11)	0.648
Brown/Roasted	8.28(0.2)	7.87(0.22)	0.153
Bloody/Serumy	1.29(0.09)	1.68(0.11)	0.005
Fat-like flavour	0.72(0.05)	0.8(0.06)	0.374
Liver-like flavour	0.14(0.02)	0.22(0.02)	0.018
Sulphur-like flavour	0.02(0.01)	0.05(0.01)	0.096
Aftertaste	3.94(0.15)	4.21(0.16)	0.239

[1]Sensory attributes were evaluated on a 15 -point descriptive scale.

[2]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.05$.

Table 3.4: Effects of post mortem ageing on the least squares means (and standard errors) of sensory attributes of the m. *longissimus thoracis*

Sensory attributes[1]	Ageing		P value[2]
	Day 3	Day 12	
n	47	47	
Overall Tenderness	9.02(0.19)	10.62(0.19)	<0.00001
Juiciness	7.62(0.18)	8.10(0.18)	0.025
Beef Flavour Identity	8.75(0.11)	8.98(0.11)	0.168
Brown/Roasted	7.89(0.18)	8.26(0.18)	0.114
Bloody/Serumy	1.50(0.10)	1.47(0.10)	0.893
Fat-like flavour	0.71(0.05)	0.81(0.05)	0.305
Liver-like flavour	0.19(0.02)	0.16(0.02)	0.433
Sulphur-like flavour	0.05(0.01)	0.02(0.01)	0.043
Aftertaste	4.01(0.15)	4.13(0.15)	0.618

[1]Sensory attributes were evaluated on a 15 -point descriptive scale.

[2]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.05$.

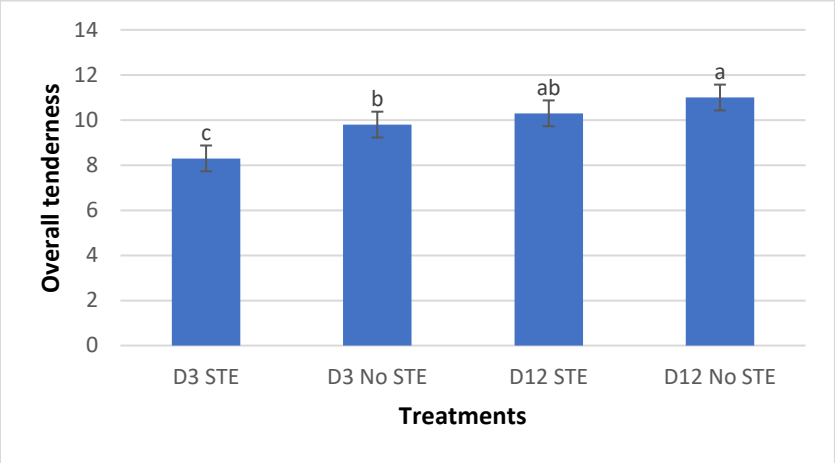

Figure 3.1: Interaction effect between steroid (STE) and ageing (D3, day 3 post mortem; D12, day 12 post mortem) for overall tenderness for the LT (P = 0.02).

[a, b, c] Treatments with a different superscript are significantly different ($P < 0.05$)

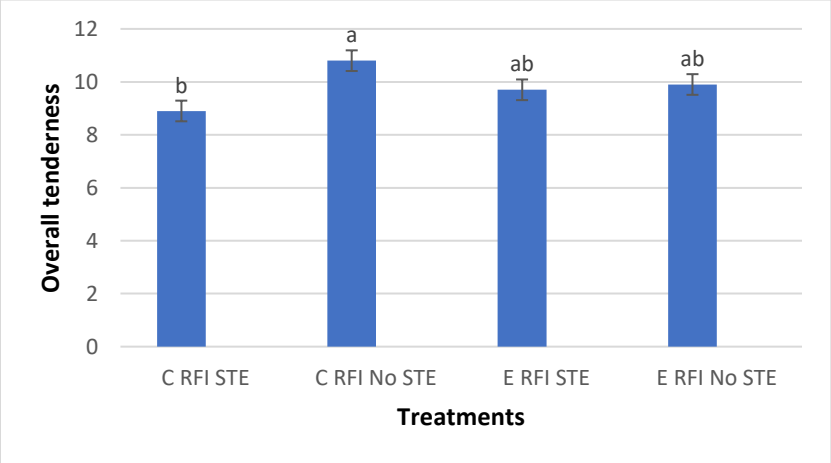

Figure 3.2: Interaction effect between RFI (C, control/high RFI; E, efficient/low RFI) and steroid (STE) for overall tenderness for the LT (P = 0.02).

[a, b] Treatments with a different superscript are significantly different ($P < 0.05$)

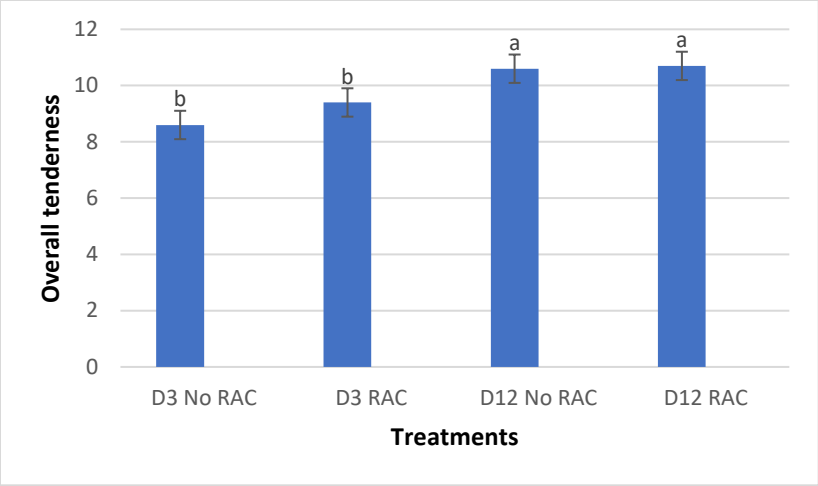

Figure 3.3: Interaction effect between RAC (No, RAC) and ageing (D3, day 3 post mortem; D12, day 12 post mortem) for overall tenderness for the LT (P = 0.039).

[a, b] Treatments with a different superscript are significantly different ($P < 0.05$)

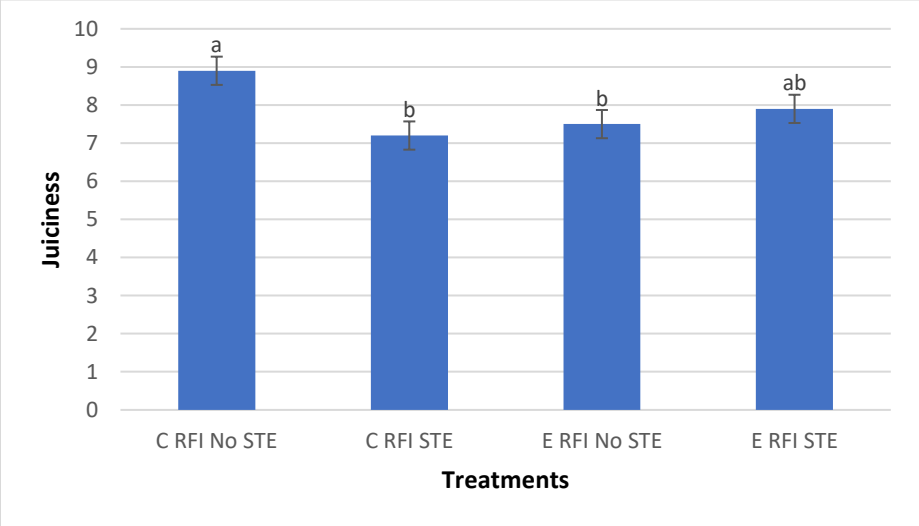

Figure 3.4: Interaction effect between RFI (C, control/high RFI; E, efficient/low RFI) and steroid (STE) for juiciness for the LT (P = 0.0007).

[a, b] Treatments with a different superscript are significantly different ($P < 0.05$)

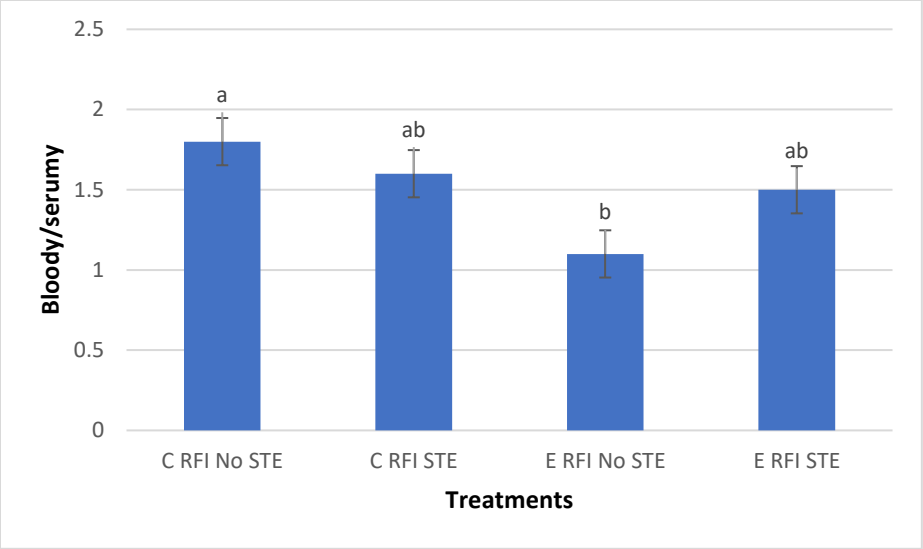

Figure 3.5: Interaction effect between RFI (C, control/high RFI; E, efficient/low RFI) and steroid (STE) for bloody/serumy for the LT (P = 0.0289).

[a, b] Treatments with a different superscript are significantly different ($P < 0.05$)

3.2 M. gluteus medius

For initial tenderness, a significant interaction existed between steroid use and ractopamine use (P = 0.028) (Figure 3.6), where the GM of steers that received no steroids and no ractopamine had a higher mean initial tenderness score than GM of steers that received steroids only, suggesting that steroids had a greater negative impact on initial tenderness score when used without ractopamine. Mean initial tenderness score of the GM was reduced by steroid use, as was the score for overall tenderness (Table 3.5), while ractopamine and selection for residual feed intake had no effects (Tables 3.6 and 3.7). Post mortem ageing increased the level of initial and overall tenderness perceived (Tables 3.8).

For juiciness, two significant three-way interactions existed. The first, between steroid use, ractopamine use and residual feed intake (P = 0.028) (Figure 3.7), although significant, showed no differences between its means. The second, between steroid use, residual feed intake and post mortem ageing (P = 0.016) (Figure 3.8) indicated that sensory juiciness scores for the GM from high RFI steers not treated with steroids was lower at day 3 than at day 12, indicating that this was the only treatment that showed an increase in juiciness with ageing (Figure 3.8).

There was no effect of steroid treatment, ractopamine supplementation, selection for residual feed intake or post mortem ageing on perceived mean beef flavour intensity or bloody/serumy scores.

For brown/ roasted flavour, the significant three-way interaction between steroid use, ractopamine use and residual feed intake (P = 0.036) (Figure 3.10) indicated that there were no differences in mean sensory scores between the treatments. The significant interaction appeared to arise from the mean brown/roasted scores being numerically higher for GM from carcasses of high RFI steers with no steroids no RAC or steroids and RAC than from similar steers that were low RFI, while there was no effect of RFI selection on steers with no steroids but treated with RAC, although brown/roasted flavour decreased in steers treated with steroids but no RAC if they were high rather than low RFI.

For fat-like, there was an interaction between steroid use, residual feed intake and post mortem ageing (P = 0.021) (Figure 3.10), although there were no differences between the means within this interaction, the main effects (Tables 3.5 to 3.8) or any other interaction. A significant interaction also existed for aftertaste between steroid and ractopamine use (P = 0.04) (Figure 3.11), but there were again no differences between the means, and no significance of the main effects (Tables 3.5 to 3.8) or any other interaction. There were no effects of residual feed intake and post mortem ageing on the perception of aftertaste flavour by panelists (Tables 3.7 and 3.8), nor any other significant interactions.

Mean panelist scores for beef liver-like and sulphur-like were unaffected by treatment of cattle with steroids (Table 3.5), ractopamine (Table 3.6), selection for residual feed intake (Table 3.7) or post mortem ageing (Table 3.8).

Table 3.5: Effects of steroids on the least squares means (and standard errors) of sensory attributes of the m. *gluteus medius*

Sensory attributes[1]	Steroid		P value[2]
	Treated	Control	
n	23	24	
Initial Tenderness	8.68(0.14)	9.10(0.12)	0.017
Overall Tenderness	9.95(0.18)	10.70(0.18)	0.005
Juiciness	8.08(0.17)	8.36(0.17)	0.173
Beef Flavour Identity	7.42(0.05)	7.42(0.05)	0.867
Brown/Roasted	7.14(0.09)	7.05(0.09)	0.505
Bloody/Serumy	1.13(0.08)	1.23(0.08)	0.437
Fat-like flavour	0.84(0.09)	0.92(0.08)	0.587
Liver-like flavour	0.82(0.08)	0.93(0.08)	0.371
Sulphur-like flavour	0.69(0.09)	0.81(0.09)	0.431
Aftertaste	4.56(0.03)	4.59(0.04)	0.652

[1]Sensory attributes were evaluated on a 15-point descriptive scale.

[2]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.05$.

Table 3.6: Effects of ractopamine on the least squares means (and standard errors) of sensory attributes of the m. *gluteus medius*

Sensory attributes[1]	Ractopamine		P value[2]
	Treated	Control	
n	22	25	
Initial Tenderness	8.88(0.14)	8.91(0.12)	0.867
Overall Tenderness	10.20(0.18)	10.40(0.18)	0.527
Juiciness	8.15(0.17)	8.30(0.16)	0.664
Beef Flavour Identity	7.45(0.05)	7.39(0.05)	0.389
Brown/Roasted	7.11(0.09)	7.08(0.09)	0.972
Bloody/Serumy	1.10(0.09)	1.25(0.08)	0.267
Fat-like flavour	0.82(0.09)	0.93(0.08)	0.355
Liver-like flavour	0.84(0.08)	0.91(0.08)	0.458
Sulphur-like flavour	0.71(0.09)	0.79(0.09)	0.543
Aftertaste	4.59(0.04)	4.56(0.03)	0.487

[1]Sensory attributes were evaluated on a 15-point descriptive scale.

[2]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.05$.

Table 3.7: Effects of residual feed intake on the least squares means (and standard errors) of sensory attributes of the m. *gluteus medius*

Sensory attributes[1]	Residual Feed Intake		P value[2]
	Efficient	Control	
n	26	21	
Initial Tenderness	8.90(0.12)	8.88(0.14)	0.998
Overall Tenderness	10.32(0.17)	10.28(0.19)	0.933
Juiciness	8.33(0.16)	8.11(0.18)	0.481
Beef Flavour Identity	7.39(0.05)	7.45(0.05)	0.411
Brown/Roasted	6.99(0.09)	7.20(0.1)	0.143
Bloody/Serumy	1.26(0.08)	1.10(0.09)	0.220
Fat-like flavour	0.90(0.08)	0.86(0.09)	0.787
Liver-like flavour	0.89(0.07)	0.84(0.08)	0.669
Sulphur-like flavour	0.78(0.09)	0.72(0.1)	0.654
Aftertaste	4.57(0.03)	4.58(0.04)	0.870

[1]Sensory attributes were evaluated on a 15-point descriptive scale.

[2]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.05$.

Table 3.8: Effects of post mortem ageing on the least squares means (and standard errors) of sensory attributes of the m. *gluteus medius*

Sensory attributes[1]	Ageing		P value[2]
	Day 3	Day 12	
n	47	47	
Initial Tenderness	8.68(0.10)	9.10(0.10)	<0.0001
Overall Tenderness	10.00(0.14)	10.60(0.13)	<0.0001
Juiciness	8.13(0.13)	8.31(0.13)	0.207
Beef Flavour Identity	7.43(0.05)	7.40(0.05)	0.699
Brown/Roasted	7.05(0.08)	7.14(0.07)	0.148
Bloody/Serumy	1.17(0.06)	1.19(0.06)	0.702
Fat-like flavour	0.89(0.06)	0.87(0.06)	0.602
Liver-like flavour	0.85(0.06)	0.89(0.06)	0.176
Sulphur-like flavour	0.74(0.06)	0.75(0.06)	0.618
Aftertaste	4.53(0.04)	4.62(0.03)	0.130

[1]Sensory attributes were evaluated on a 15-point descriptive scale.

[2]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.05$.

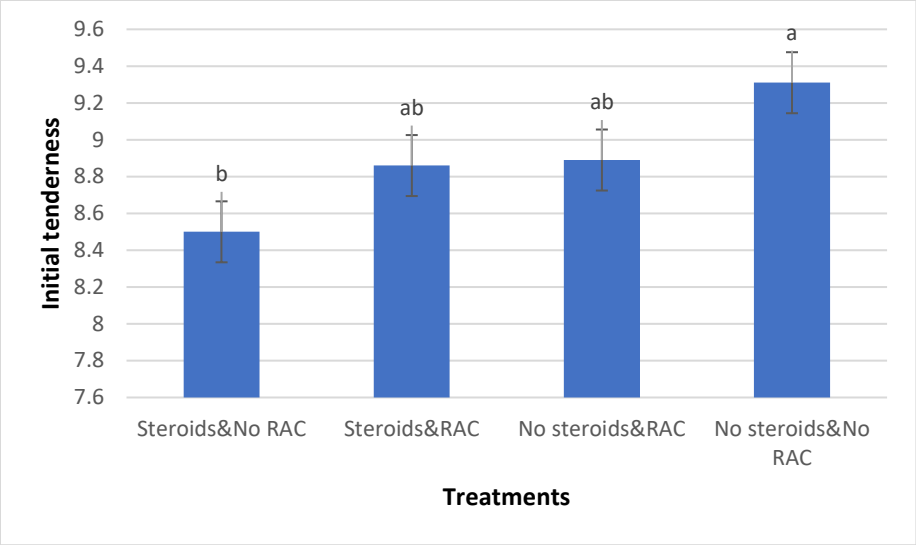

Figure 3.6: Interaction between RAC (No RAC, RAC) and steroid use (No steroid, steroid) for initial tenderness of the GM (P = 0.028).

[a, b] Treatments with a different superscript are significantly different ($P < 0.05$)

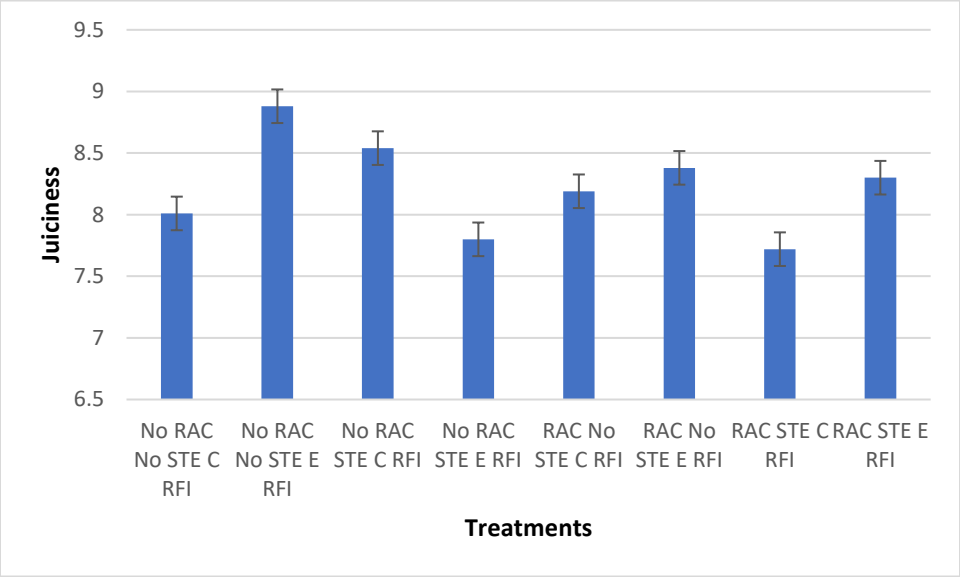

Figure 3.7: Interaction between RFI (C, control/high RFI; E, efficient/low RFI), steroid use (No STE, STE) and RAC (No RAC, RAC) on sensory juiciness scores for the GM (P = 0.028).

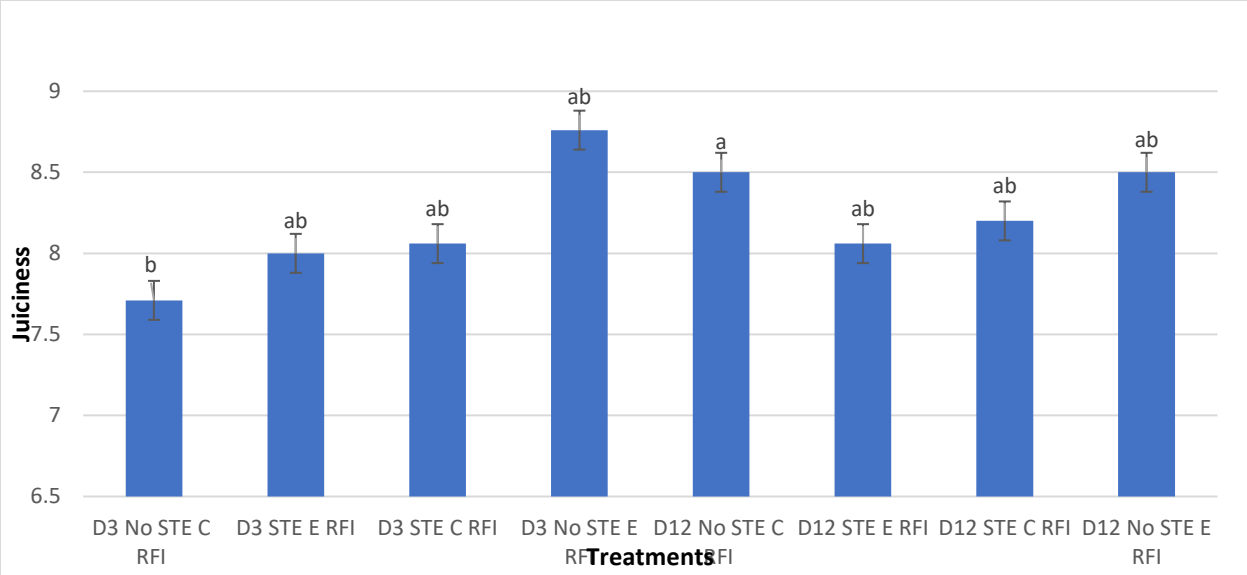

Figure 3.8: Interaction between RFI (C, control/high RFI; E, efficient/low RFI), steroid use (No STE, STE) and ageing (D3, day 3 post mortem; D12, day 12 post mortem) for sensory juiciness scores of the GM (P = 0.016).

[a, b] Treatments with a different superscript are significantly different ($P < 0.05$)

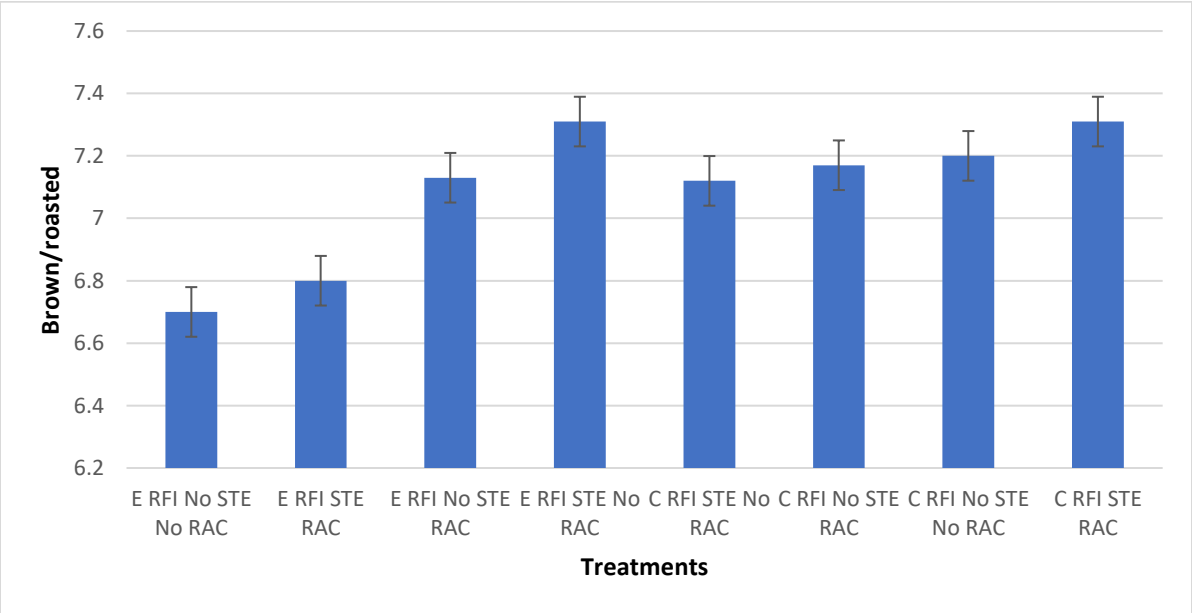

Figure 3.9: Interaction between RFI, steroid use and RAC for brown/roasted of GM (P value = 0.036).

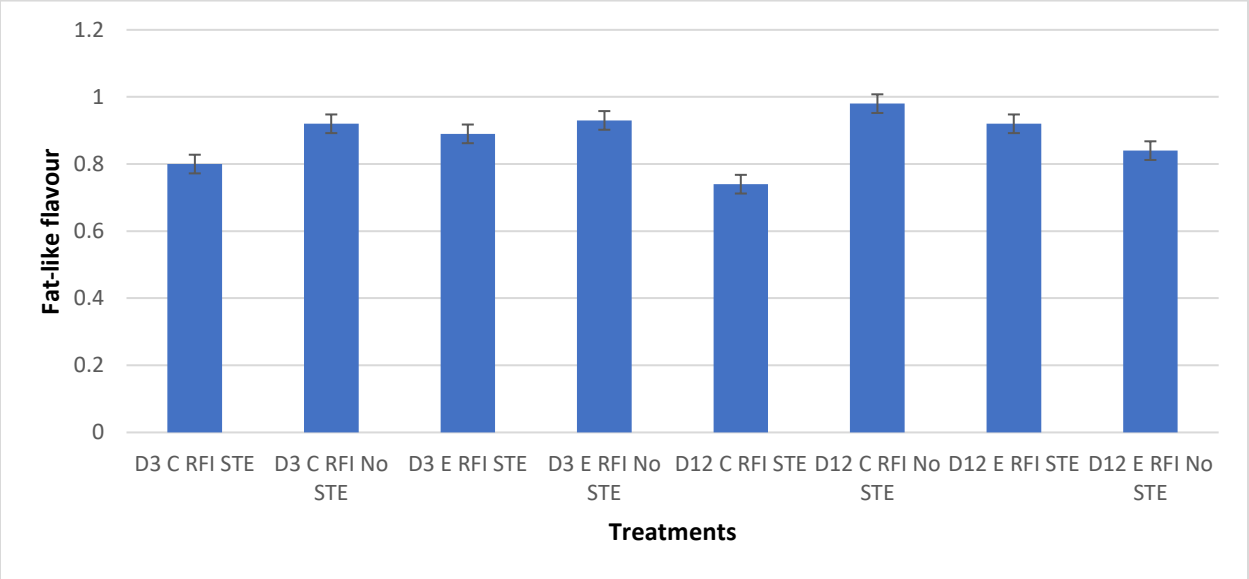

Figure 3.10: Interaction between RFI (C, control/high RFI; E, efficient/low RFI), steroid (No STE, STE) and ageing (D3, day 3 post mortem; D12, day 12 post mortem) for Fat-like flavour scores of the GM (P = 0.021).

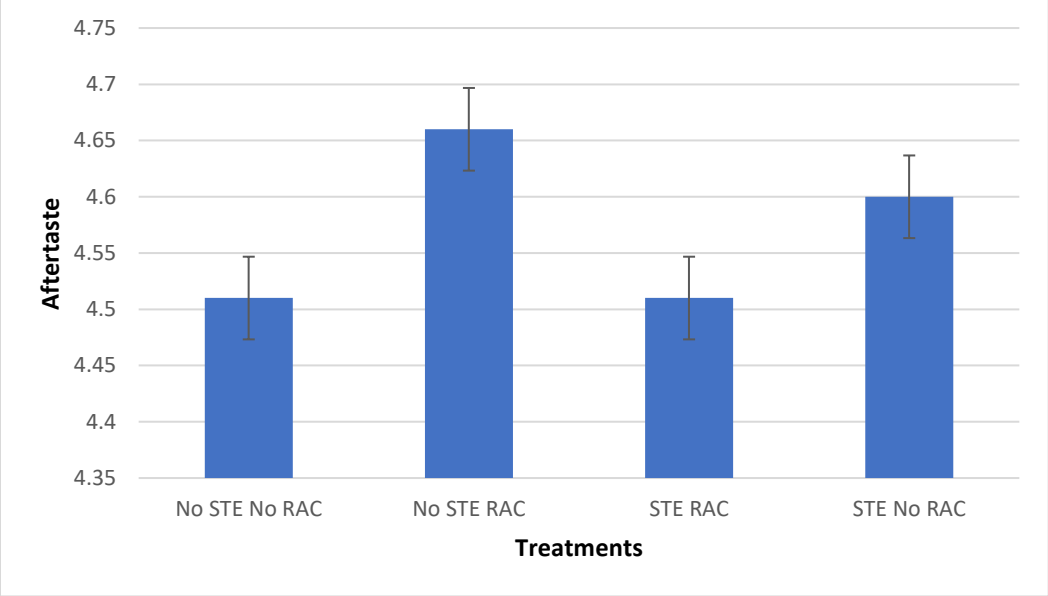

Figure 3.11: Interaction between steroid and RAC for aftertaste of the GM (P = 0.04).

3.3 M. semimembranosus

For initial tenderness, a significant interaction existed between ractopamine use and post mortem ageing (P = 0.001) (Figure 3.12). The interaction between ractopamine status and post mortem ageing indicated that the mean initial tenderness scores for the SM increased with ageing when the steers did not receive ractopamine and did not change when the steers did receive ractopamine (Figure 3.12). Mean initial and overall tenderness scores of the SM decreased with steroid use (Table 3.9) but were unaffected by supplementation with ractopamine (Table 3.10) or selection of steers for low RFI (Table 3.11), and there were no other interactions.

Mean juiciness rating was also affected by steroid use, with SM from carcasses from steers that received steroids having a lower score that those not treated (P = 0.004) (Table 3.9). Juiciness sensory scores were unaffected by ractopamine supplementation, selection for RFI, and post mortem ageing (Tables 3.9 to 3.12).

For bloody/serumy flavour, there was an interaction between residual feed intake and post mortem ageing (P = 0.044), although the treatment means within the interaction were not different. The interaction was driven by the mean scores for bloody/serumy flavour being numerically higher with ageing in SM from carcasses of low RFI cattle but being numerically lower in that from high RFI steers (Figure 3.13). Bloody/serumy flavour was not affected by any other interactions, nor was it affected by steroid use (Table 3.9), ractopamine use (Table 3.10), selection for residual feed intake (Table 3.11) or post mortem ageing (Table 3.12).

Residual feed intake and post mortem ageing interacted (P = 0.004) for the attribute fat-like, with mean panelist scores for this attribute decreasing for SM from the carcasses of high RFI steers with ageing while SM from low RFI steers did not change (Figure 3.14). There were no effects of ractopamine (Table 3.10) or steroid use (Table 3.9) on the perception of fat-like by panelists, nor any other significant interactions for this attribute.

There was an interaction effect between ractopamine and residual feed intake for liver-like flavour (P = 0.04). No differences between the treatment means for this interaction were observed (Figure 3.15), nor were there effects of steroid use and post mortem ageing on the perception of liver-like, or any other significant interactions.

Sulphur-like was increased and after-taste decreased in SM from the carcasses of steers treated with steroids (Table 3.9), but these attributes were unaffected by supplementation of ractopamine (Table 3.10), selection for RFI (Table 3.11) and ageing (Table 3.12). Mean panelist scores for beef flavour intensity and brown/roasted flavour were unaffected by treatment of cattle with steroids (Table 3.9), ractopamine (Table 3.10), selection for residual feed intake (Table 3.11) or post mortem ageing (Table 3.12).

3.4 Effects of steroid, ractopamine, RFI and post mortem ageing on purge loss, cook loss and yield percentage of all muscles

Implanting the cattle with steroids did not have any significant effects on purge loss, cook loss and yield percentage for LT, GM and SM (Table 3.13). Feeding RAC to the cattle also did not have any significant effects ($P>0.05$) on purge loss, cook loss and yield percentage in the muscles studied (Table 3.14). The influence of RAC on purge loss of the LT muscle approached significance ($P=0.080$) with LT from cattle supplemented with RAC tending to have greater purge loss than control cattle (Table 3.14). RFI status also did not affect purge loss percentage ($P > 0.05$) in any of the muscles studied, but it increased cook loss and decreased yield percentage in the LT muscle ($P = 0.010$) (Table 3.15). Mean cook losses and yield percentages for the GM and SM muscles were not affected ($P > 0.05$) by RFI. Post mortem ageing had an effect on purge loss percentage of the GM and SM muscles (Table 3.16), with meat samples that were aged 3 days post mortem having greater purge loss than meat samples that were aged for 12 days post mortem in both muscles (Table 3.16). The effect of ageing on the cook loss and yield percentages of the LT did approach significance ($P = 0.064$), with mean cook loss percentage of day 12 LT tending to be greater than that of day 3 LT and the yield percentage tending to decrease with ageing time (Table 3.16). However, post mortem ageing did not have any significant effects ($P > 0.05$) on cook loss and yield percentage of the LT, GM and SM muscles.

Table 3.9: Effects of steroids on the means (and standard errors) of sensory attributes of the m. *semimembranosus*

Sensory attributes[1]	Steroid		P value[2]
	Treated	Control	
n	23	24	
Initial Tenderness	7.08(0.11)	7.80(0.11)	<0.0001
Overall Tenderness	8.68(0.13)	9.46(0.13)	<0.0001
Juiciness	7.17(0.11)	7.60(0.11)	0.004
Beef Flavour Identity	7.46(0.06)	7.60(0.06)	0.085
Brown/Roasted	7.61(0.11)	7.73(0.11)	0.403
Bloody/Serumy	1.18(0.03)	1.22(0.03)	0.332
Fat-like flavour	1.16(0.03)	1.17(0.03)	0.886
Liver-like flavour	1.07(0.02)	1.09(0.02)	0.604
Sulphur-like flavour	1.03(0.01)	1.00(0.01)	0.038
Aftertaste	4.59(0.05)	4.72(0.05)	0.050

[1]Sensory attributes were evaluated on a 15-point descriptive scale.

[2]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.05$.

Table 3.10: Effects of ractopamine on the means (and standard errors) of sensory attributes of the m. *semimembranosus*

Sensory attributes[1]	Ractopamine		P value[2]
	Treated	Control	
n	22	25	
Initial Tenderness	7.40(0.11)	7.49(0.11)	0.697
Overall Tenderness	8.98(0.13)	9.16(0.13)	0.429
Juiciness	7.43(0.11)	7.34(0.11)	0.512
Beef Flavour Identity	7.50(0.06)	7.55(0.06)	0.455
Brown/Roasted	7.68(0.11)	7.66(0.11)	0.833
Bloody/Serumy	1.20(0.03)	1.19(0.03)	0.819
Fat-like flavour	1.15(0.03)	1.18(0.03)	0.587
Liver-like flavour	1.06(0.02)	1.10(0.02)	0.211
Sulphur-like flavour	1.01(0.01)	1.02(0.01)	0.355
Aftertaste	4.66(0.05)	4.65(0.05)	0.893

[1]Sensory attributes were evaluated on a 15-point descriptive scale.

[2]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.05$.

Table 3.11: Effects of residual feed intake on the means (and standard errors) of sensory attributes of the m. *semimembranosus*

Sensory attributes[1]	Residual Feed Intake		P value[2]
	Efficient	Control	
n	26	21	
Initial Tenderness	7.43(0.11)	7.45(0.11)	0.894
Overall Tenderness	9.07(0.12)	9.07(0.14)	0.987
Juiciness	7.47(0.10)	7.30(0.12)	0.287
Beef Flavour Identity	7.50(0.06)	7.56(0.06)	0.465
Brown/Roasted	7.63(0.10)	7.71(0.11)	0.597
Bloody/Serumy	1.21(0.03)	1.18(0.03)	0.457
Fat-like flavour	1.19(0.03)	1.14(0.03)	0.258
Liver-like flavour	1.09(0.02)	1.08(0.02)	0.726
Sulphur-like flavour	1.01(0.01)	1.02(0.01)	0.398
Aftertaste	4.63(0.05)	4.68(0.05)	0.554

[1]Sensory attributes were evaluated on a 15-point descriptive scale.

[2]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.05$.

Table 3.12: Effects of post mortem ageing on the means (and standard errors) of sensory attributes of the m. *semimembranosus*

Sensory attributes[1]	Ageing		P value[2]
	Day 3	Day 12	
n	47	47	
Initial Tenderness	7.19(0.09)	7.69(0.09)	<0.0001
Overall Tenderness	8.80 (0.10)	9.34 (0.10)	<0.0001
Juiciness	7.41(0.09)	7.36(0.09)	0.699
Beef Flavour Identity	7.50(0.06)	7.55(0.06)	0.574
Brown/Roasted	7.57(0.09)	7.77(0.09)	0.093
Bloody Serumy	1.20(0.03)	1.20(0.03)	0.696
Fat-like flavour	1.18(0.03)	1.15(0.03)	0.456
Liver-like flavour	1.07(0.02)	1.09(0.02)	0.414
Sulphur-like flavour	1.01(0.01)	1.01(0.01)	1.000
Aftertaste	4.60(0.05)	4.71(0.05)	0.164

[1]Sensory attributes were evaluated on a 15-point descriptive scale.

[2]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.05$.

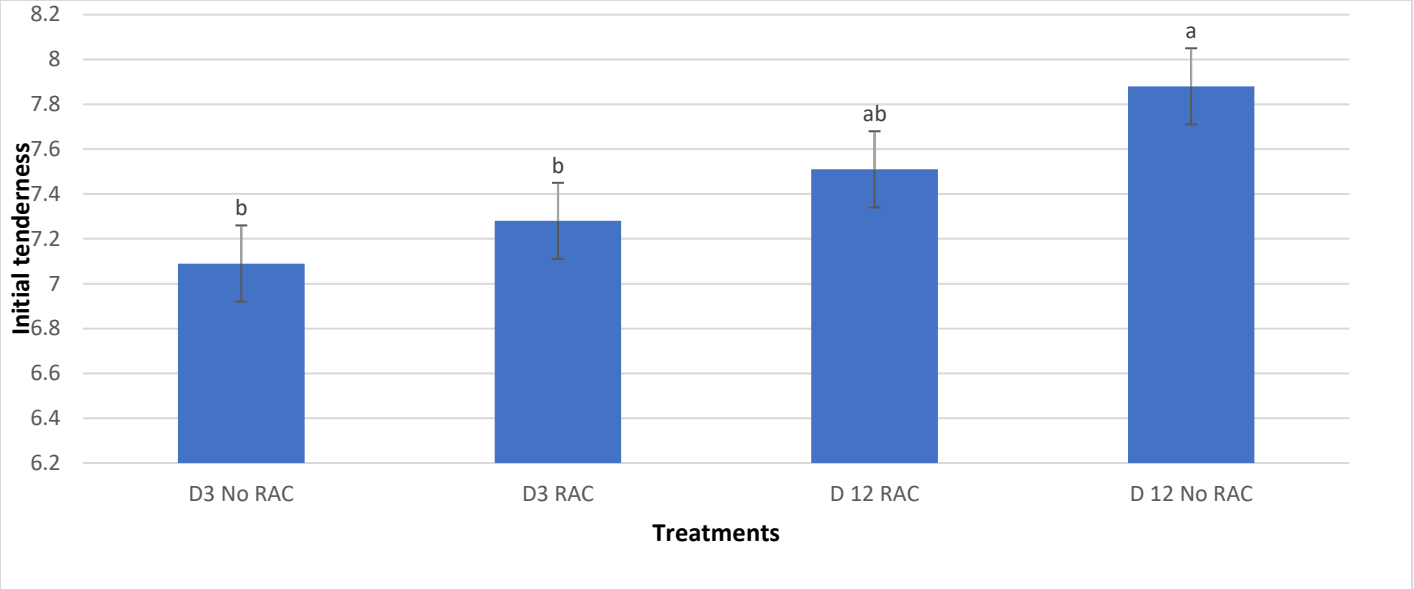

Figure 3.12 Interaction between RAC and ageing for initial tenderness of the SM (P = 0.001).

[a, b] Treatments with a different superscript are significantly different ($P < 0.05$)

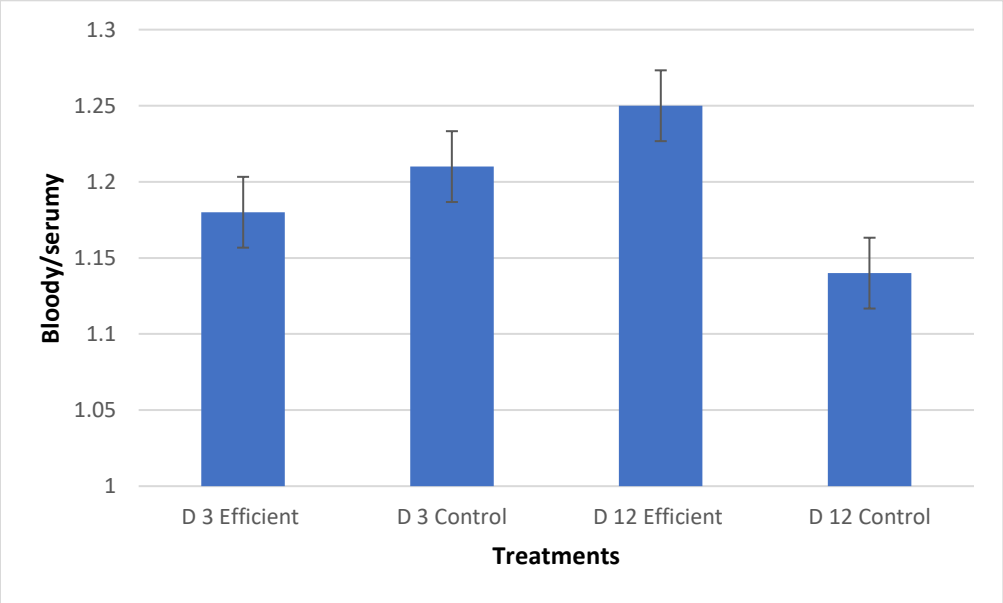

Figure 3.13 Interaction between residual feed intake and ageing for bloody/serumy of the SM (P = 0.044)

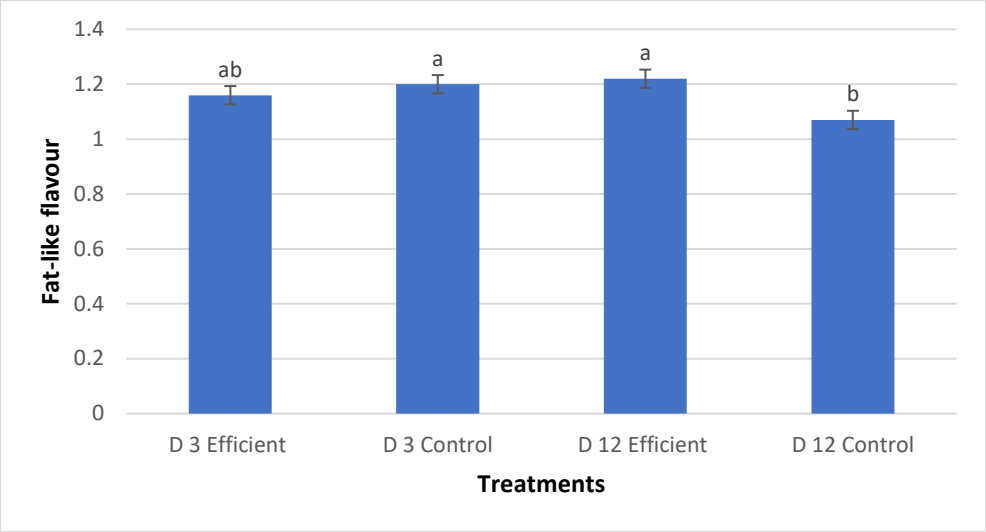

Figure 3.14 Interaction between residual feed intake (Control/high RFI, Efficient/low RFI) and ageing (D3, day 3 post mortem; D12, day 12 post mortem) for Fat-like flavour of the SM (P = 0.004).

[a, b] Treatments with a different superscript are significantly different ($P < 0.05$)

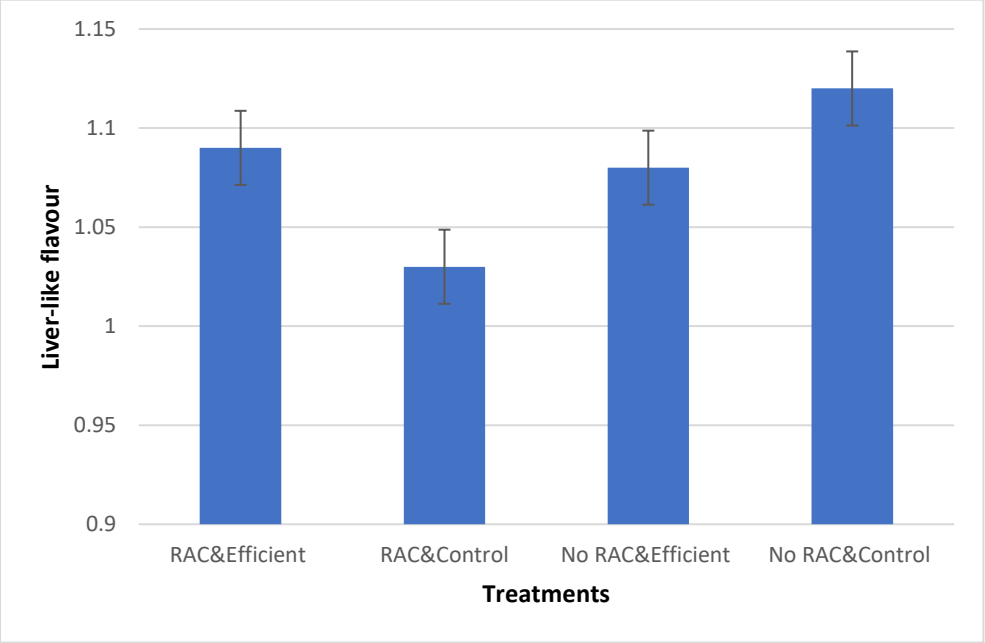

Figure 3.15 Interaction between RFI (Control/high RFI, Efficient/low RFI) and RAC (No RAC, RAC) for liver-like flavour of the SM (P = 0.04)

Table 3.13: Effects of steroid on the means (and standard errors) of purge loss, cook loss and yield percentages of the m. *longissimus thoracis*, m. *gluteus medius* and m. *semimembranosus.*

Variables	Muscles								
	m. *longissimus thoracis*			m. *gluteus medius*			m. *semimembranosus*		
	Treated	Control	P value[1]	Treated	Control	P value[1]	Treated	Control	P value[1]
n	23	24		23	24		23	24	
Purge loss (%)	3.52 (0.37)	3.96 (0.37)	0.290	5.86 (0.34)	6.07 (0.34)	0.883	7.06 (0.62)	7.68 (0.64)	0.552
Cook loss (%)	18.90 (0.60)	18.70 (0.59)	0.903	22.10 (0.64)	22.20 (0.64)	0.776	28.80 (0.61)	28.50 (0.60)	0.524
Yield (%)	81.10 (0.60)	81.30 (0.59)	0.903	77.90 (0.64)	77.80 (0.64)	0.776	71.20 (0.61)	71.50 (0.60)	0.524

[1]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.05$.

Table 3.14: Effects of ractopamine on the means (and standard errors) of purge loss, cook loss and yield percentages of the m. *longissimus thoracis*, m. *gluteus medius* and m. *semimembranosus.*

Variables	Muscles								
	m. *longissimus thoracis*			m. *gluteus medius*			m. *semimembranosus*		
	Treated	Control	P value[1]	Treated	Control	P value[1]	Treated	Control	P value[1]
n	22	25		22	25		22	25	
Purge loss (%)	4.19 (0.37)	3.30 (0.37)	0.080	5.97 (0.33)	5.95 (0.34)	0.781	6.65 (0.63)	8.09 (0.63)	0.110
Cook loss (%)	18.70 (0.60)	18.90 (0.59)	0.916	22.40 (0.64)	21.80 (0.64)	0.715	28.10 (0.61)	29.20 (0.60)	0.172
Yield (%)	81.30 (0.60)	81.10 (0.59)	0.916	77.60 (0.64)	78.20 (0.64)	0.715	71.90 (0.61)	70.80 (0.60)	0.172

[1]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.05$.

Table 3.15: Effects of residual feed intake on the means (and standard errors) of purge loss, cook loss and yield percentages of the m. *longissimus thoracis*, m. *gluteus medius* and m. *semimembranosus.*

Variables	Muscles								
	m. *longissimus thoracis*			m. *gluteus medius*			m. *semimembranosus*		
	Efficient	Control	P value[1]	Efficient	Control	P value[1]	Efficient	Control	P value[1]
n	26	21		26	21		26	21	
Purge loss (%)	3.44 (0.35)	4.05 (0.39)	0.262	5.72 (0.32)	6.21 (0.35)	0.412	7.95 (0.60)	6.79 (0.66)	0.238
Cook loss (%)	19.90 (0.56)	17.70 (0.62)	0.010	22.30 (0.62)	22.00 (0.67)	0.680	28.20 (0.58)	29.10 (0.64)	0.241
Yield (%)	80.10 (0.56)	82.30 (0.62)	0.010	77.70 (0.62)	78.00 (0.67)	0.680	71.80 (0.58)	70.90 (0.64)	0.241

[1]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.05$.

Table 3.16: Effects of post mortem ageing on the means (and standard errors) of purge loss, cook loss and yield percentages of the m. longissimus thoracis, m. gluteus medius and m. semimembranosus.

Variables	Muscles								
	m. *longissimus thoracis*			m. *gluteus medius*			m. *semimembranosus*		
	Day 3	Day 12	P value[1]	Day 3	Day 12	P value[1]	Day 3	Day 12	P value[1]
n	47	47		47	47		47	47	
Purge loss (%)	3.94 (0.38)	3.55 (0.36)	0.526	6.80 (0.30)	5.12 (0.30)	0.0001	8.60 (0.51)	6.14 (0.50)	<.0001
Cook loss (%)	18.10 (0.54)	19.50 (0.54)	0.064	22.30 (0.63)	22.00 (0.62)	0.853	28.60 (0.51)	28.70 (0.51)	0.964
Yield (%)	81.90 (0.54)	80.50 (0.54)	0.064	77.70 (0.63)	78.00 (0.62)	0.853	71.40 (0.51)	71.30 (0.51)	0.964

[1]P value is the probability of the null hypothesis being true, with the null hypothesis rejected at $P < 0.0$

CHAPTER 4: Discussion

4.1 Steroids and beef tenderness

Tenderness is one of the most important factors affecting consumers' decision to purchase beef, and studies have shown that consumers are willing to pay more for "guaranteed tender" steak (Dransfield, 1998; Lusk et al., 2001). Factors influencing meat tenderness can impact overall consumer acceptability of beef products (Lusk et al., 2001; O'Quinn et al. 2018). Several studies have indicated that beef tenderness is the most important factor influencing consumer satisfaction (Savell et al., 1987; Miller et al., 2001), although recently this has been disputed (O'Quinn et al. 2018).

The use of anabolic implants has a long-standing place in the cattle feeding industry due to their positive impact on growth performance and subsequent profitability. Implantation of cattle with steroid growth promotants is common in the beef industry (Samuelson et al., 2016), and combination hormone implants containing both estradiol and trenbolone acetate can increase growth rates by 20% and improve feed efficiency by 15% compared with cattle not receiving hormone implants (Schanbacher, 1984; Bartle et al., 1992; Johnson et al., 1996). However, implants can have adverse effects on carcass quality by reducing marbling, increasing shear force, and decreasing eating quality depending on the type of hormone, its dose and the frequency of implantation, or what some refer to as the aggressiveness of the implant regimen administered (Garmyn and Miller 2014). Some implanting strategies reduced the eating quality and consumer satisfaction of beef specifically by increasing the WBSF and the occurrence of tough beef (Samber et al., 1996; Foutz et al., 1997). Ebarb et al. (2016) and Packer et al. (2019) concluded that steroid-treated animals had tougher meat than control cattle prior to ageing. Ebarb et al. (2016) found the reduction in tenderness caused by growth promoters was due to increased muscle fiber cross-sectional area, and that collagen solubility was not affected by growth promoter treatment. Platter et al. (2003) reported that repeated implanting not only increased growth, but also had detrimental effects on carcass quality by decreasing m. *longissimus thoracis* marbling score, increasing WBSF values, and decreasing consumer taste panel scores for tenderness like/dislike. Previous literature demonstrated that growth promoters negatively impacted tenderness through increased calpastatin activity (Gerken et al. 1995; Strydom et al. 2009). In contrast, Belk and Savell (1992) concluded that use of TBA and estradiol implants did

not affect beef tenderness, while Gerken et al. (1995) recorded that combined TBA and estradiol implants had no effect on WBSF values of strip loin steaks. Barham et al. (2003) also concluded that WBSF values were not affected by an implant regimen, which consisted of 2 estrogenic implants or one estrogenic implant followed by a high-potency combination implant. Lean et al. (2018) concluded that using HGP did not have any negative/reduced impact on meat tenderness, and they further went on to say that using multiple HGP improved meat tenderness, compared to using a single implant, and that is in contrast to the findings from this study, where HGP did have a negative effect on beef tenderness. Clearly, questions remain about the effects of HGP on beef quality, particularly on measures of toughness such as WBSF, and sensory attributes such as tenderness, juiciness, flavour, and connective tissue (Watson, 2008).

In this thesis, the steers were implanted twice: once with Component E-S followed by once with Component TE-S. Results from this study showed that implanted steers had reduced tenderness across all muscles studied, and this contrasts with Igo et al (2011), who concluded that implanted steaks did not differ from non-implanted steaks, as no difference in WBSF values was recorded. Evaluation of beef toughness using WBSF does not necessarily represent sensory perception of tenderness, however, as assessments of toughness by WBSF and trained sensory panels often show poor correlations depending upon the muscle (Shackelford et al. 1995).

The differences in the effects of steroids on meat quality across studies may be due to the types and numbers of steroids used in this study. Duckett and Pratt (2014) submitted that the increase in WBSF they observed with repeated treatments with HGP was associated with androgenic rather than estrogenic steroids. The study of Duckett and Pratt (2014) was similar to this study, as the steers were implanted twice and a decrease in mean tenderness also was observed for the implanted steers. Packer et al. (2018), however, found that even estrogenic implants can increase WBSF and decrease sensory tenderness, juiciness and overall liking. From the sensory tenderness results in this thesis, we can conclude that the trained panelists were able to observe a difference in the tenderness of steaks between animals treated with both estrogenic and androgenic HGP and those from steers that were controls. Barham et al. (2003) concluded that implanting cattle did not affect consumer evaluations of beef tenderness after 7 and 14 days post mortem ageing, results that contrast with those of this thesis where reduced tenderness ratings were recorded by trained panellists for the LT, GM and SM from the carcasses of implanted

cattle regardless of post mortem ageing. With no significant interaction between post mortem ageing and steroid application, the decrease in tenderness scores with steroid use persisted in the present study.

Finally, the results from this thesis and that of Ebarb et al. (2017) suggest that implanted cattle will have a lower tenderness rating compared to non-implanted cattle, despite other authors having reported no differences in consumer tenderness scores for muscles from cattle that received an aggressive implant during growth (Barham et al. 2003). Further research is warranted that addresses the implications of hormone type, post mortem ageing duration, and breed on the impact of steroids on consumer perceptions of beef tenderness, juiciness and flavour.

Steroids reduced the sensory ratings for juiciness and increased sulphur-like and reduced after-taste attributes in the SM. The results in this thesis differ from those of Cranwell et al. (1996) who found that trenbolone acetate increased sensory juiciness of LT from re-fed thin cows. They also differ from those of Barham et al. (2003) who found that juiciness of the m. longissimus lumborum (LL) was also increased in steers administered steroids. Beef flavour is a balance between fat content and fatty acid concentration in the fat, and changes in fatty acids and meat flavour have been observed between rams and wethers (Vesely 1973). Steroids may have reduced juiciness ratings in the study described in this thesis by reducing intramuscular fat (Lucherk et al. 2016) in the SM, although this was not confirmed. Barham et al. (2003) also used both trained and consumer panels to assess the impact of steroids on beef flavour and found no effect of steroid. Igo et al. (2011), however, found that steroids reduced beef flavour intensity and overall acceptability in beef aged for 14 days using a consumer panel, but this difference disappeared by 21 days. The results in this thesis and those of others suggest that the effect of steroids may interact with intramuscular fat content and its fatty acid profile. Further research is warranted in this area so that the flavour of beef during ageing can be stabilized and the development of undesirable off-flavours prevented.

4.2 Post mortem ageing and beef tenderness

Beef tenderness has been listed as one of the most important factors affecting consumer satisfaction (Dikeman 1987; Savell et al. 1989; O'Quinn et al. 2018). Because of this, post mortem tenderization methods such as ageing (Dikeman et al. 2013, Parrish et al. 1991, Sitz et

al. 2006) are used in the beef industry to add value to product for target markets. Ageing increases tenderness and flavour of meat (Sitz et al. 2006). It has been proposed that ageing can reduce the effects of HGP on WBSF (Thompson et al. 2008). Several experiments support this theory (Schneider et al. 2007; Thompson et al. 2008; Igo et al. 2011; Packer et al. 2018), while some do not (Platter et al. 2003).

In this thesis, the results indicated that post mortem ageing of beef samples had a positive impact on beef tenderness, as there was a significant increase in sensory tenderness ratings between 3 d and 12 d samples for the LT, GM and SM. Ebarb et al. (2017) reported that treatment and post mortem ageing influenced WBSF values, and they also went further to say that all their post mortem ageing comparisons differed from one another, indicating that as post mortem ageing time increased, shear force values of steaks decreased. Ebarb et al. (2017) reported that, over the total ageing time in their study, steaks from cattle treated with steroids had greater shear force values when compared to steaks from non-treated cattle, and this was also the same finding in this thesis where it was observed that the effect of HGP on tenderness was most prominent prior to post mortem ageing. This was observed by Boler et al. (2012) as well who observed that when steaks from cattle fed RAC were aged 4 d they had a 13% greater mean WBSF value compared to that of controls; however, after 7, 14, and 21 days post mortem, mean WBSF values did not differ. Findings from Quinn et al. (2008) and Schneider et al. (2007) indicated that samples from implanted cattle usually required up to 7 days post mortem for mean shear force values to be comparable to that of control samples. Ebarb et al. (2016) observed that steaks from implanted heifers took 14 d to reach a mean WBSF value similar to that of the controls, but steaks from heifers implanted and fed ZH did not reach control steak mean WBSF values even after 35 d of ageing. Smith et al. (2007) reported steers subjected to an implant containing 200 mg TBA and 28 mg estradiol produced steaks with 15% greater WBSF when aged over a 21 d period, a finding that is in contrary to the results from this study, as there was a positive effect of post mortem ageing on the beef from implanted cattle in this study. Savell et al. (1982) reported a positive effect of increasing post mortem storage from 4 to 18 d on sensory panel tenderness ratings; likewise, Gruber et al. (2006) reported that the rate of tenderization in the *longissimus lumborum* and *gluteus medius* decreased with increasing ageing time. In agreement with this study, George-Evins et al. (2004) found that ageing from 7 to 21 days had a positive effect on WBSF values of *gluteus medius* steaks. King et al. (2009) indicated that slice shear force of non-

blade-tenderized *gluteus medius* steaks decreased between ageing intervals. Clearly post mortem ageing decreases beef toughness, and the results in this study agree with this.

Reports regarding ageing in the *longissimus lumborum* (Smith et al., 1978; Gruber et al., 2006, 2008) and *gluteus medius* (Harris et al., 1992; Eilers et al., 1996; George et al., 1999; Gruber et al., 2006) indicated that, generally, extended ageing times will result in greater proteolysis and improved tenderness, although these changes may not be linear and may not be large enough to be statistically significant at all incremental increases in time. Obuz et al. (2014) concluded that post mortem ageing was very effective in improving sensory tenderness of cull Holstein cow *longissimus lumborum* steaks, and they also stated that ageing method and ageing time affected overall tenderness positively. Increased overall tenderness rating was also recorded by George-Evins et al. (2004) for steaks aged for 21 compared to 7 days. Similar to the result from this present study, Wheeler et al. (1999) reported decreased WBSF with an increase in ageing time. Again, the sensory results in this thesis agree with the results of these authors that post mortem ageing can profoundly increase sensory tenderness of beef.

Ageing for 12 days post mortem also increased sulphur-like flavour in the LT, but had no effect of flavours of the other muscles. The detection of sulphur-like aromas and compounds in beef is not uncommon (Macleod 1994), and such flavours are often associated with reduced palatability (Meisinger et al. 2006). Because a trained panel was used in this thesis, no measure of acceptability was made, and so whether the development of this flavour note in the LT with ageing was meaningful or not is not known.

4.3 Ractopamine and beef sensory quality

Within the last ten years, RAC has gained recognition in the beef industry, as it is used in beef finishing diets to improve growth performance and carcass yields. Efficient use of nutrients is vital for profitability and sustainability of beef cattle production. Beta adrenergic agonists (BA) are additives commonly used to increase the efficiency of animal gain in the beef industry. The efficacy of BA have been demonstrated in several studies with young, castrated *Bos taurus* cattle with a high degree of fat thickness and marbling (Gruber et al. 2007; Quinn et al. 2008; Scramlin et al. 2010). Ractopamine hydrochloride (RAC) is a beta-1 adrenergic agonist that promotes the repartitioning of nutrient flow from lipogenesis towards protein accretion (Yang and McElligott 1989). Feeding ractopamine increases average daily gain, improves feed efficiency and increases

both live and hot carcass weight (Schroeder 2004; Dunshea et al. 2005; Avendaño Reyes et al. 2006). This increase in muscle mass can be attributed to an increase in muscle protein synthesis, a reduction in protein degradation or some combination of both (Scramlin et al. 2010).

Much like anabolic implants, these repartitioning agents can have negative effects on WBSF, but the differences do not necessarily translate directly to consumer responses for palatability and acceptance particularly if tenderness is managed through proper post mortem ageing (Garmyn and Miller 2014). Rathmann et al. (2009) suggested that a BA increases the transcriptional activity of calpastatin and in turn this increase could be the reason for the reduction in tenderness observed with ractopamine supplementation (Leheska et al. 2008; Kellermeier et al. 2009). Research suggests the BA approved for cattle in the United States, specifically zilpaterol hydrochloride (ZH) and RAC, can be used to alleviate the conformation and yield challenges of calf-fed Holstein animals (Vogel et al., 2009; Lawrence et al., 2011). Both ZH and RAC have been used to increase carcass muscling, but their utilization has elicited variable and undesirable changes in meat quality traits, including increased toughness (Brooks et al. 2009; Scramlin et al. 2010; Van Donkersgoed et al. 2011). Previous literature has confirmed increased WBSF in steaks from cattle fed BA, with Woerner et al. (2011) reporting an increase in WBSF values in twice-implanted calf-fed Holsteins steers and heifers supplemented with RAC. A meta-analysis conducted by Platter and Choat (2008) suggested that RAC supplementation increased *longissimus* WBSF by an average of 0.2 kg, but Van Donkersgoed et al. (2011) detected no differences in WBSF values of *longissimus* steaks from feedlot heifers fed RAC. Gruber et al. (2008) reported increased WBSF for steers fed RAC, in agreement with the study of Van Donkersgoed et al. (2011), while Quinn et al. (2008) observed no difference in shear force values of steaks from heifers subjected to RAC and those from control heifers.

Martin et al. (2014) concluded that feeding RAC to cattle resulted in greater WBSF values than values from control, and this increase persisted even after 16 and 23 days of ageing even though the WBSF of the steaks from the RAC cattle muscles decreased with ageing. Several authors (Hilton et al., 2009; Holmer et al., 2009; Leheska et al., 2009) found that although post mortem ageing reduced the WBSF values of steaks from cattle that received BA, they were never as tender as control samples. Martin et al. (2014) concluded that regardless of BA supplement,

WBSF values were still considered tender according to American Society for Testing and Materials (ASTM) slice shear force threshold.

Several studies have shown a negative impact of BA on meat tenderness (Geesink et al., 1993; Vestergaard et al., 1994) but in the present thesis, there was no negative effect of RAC supplementation on meat tenderness as assessed by trained sensory panelists. Although the correlation between sensory tenderness and WBSF is often moderate at best (Shackelford et al., 1995), Arp et al. (2013) reported a dose response of RAC, with no difference in WBSF values for meat from steers treated with 200 mg/animal/day compared with non-treated control steers at 14 days post mortem. Quinn et al. (2008) reported that WBSF values obtained from cooked *longissimus* steak core samples were not different for heifers fed control and RAC, and the sensory tenderness assessment results of this thesis agree with the studies that used WBSF to show no profound effect of RAC on toughness of beef from cattle fed 200 mg RAC/head/day.

4.4 Residual feed intake and beef sensory quality

Current genetic selection programs are focused primarily on growth and carcass traits, which are easily and inexpensively measured. However, it is important that any process of selection for efficiency does not adversely impact improvements made in end-product quality (Archer et al., 1999). Some studies (Gomes et al., 2012, Welch et al., 2012) found no relationship between RFI and shear force in non-aged and aged steaks, while others (Herd & Pitchford, 2011) show that selection for low RFI would negatively affect meat tenderness. Zorzi et al. (2013) found low RFI bulls had a higher mean shear force value than that of high RFI bulls. McDonagh et al. (2001) reported 13% greater calpastatin activity in muscle tissue from low RFI steers compared with high RFI steers, suggesting that lower RFI steers may have decreased meat tenderness in comparison with high RFI steers, but reported no differences in shear force values of LM steaks aged for 1 or 14 d between high and low RFI steers. Baker et al. (2006) reported no difference in WBSF values among high, mid, and low RFI cattle, and went further to conclude that all steaks over the ageing periods tested from high and low RFI cattle fell within the industry standard and would be considered tender, and that trained sensory panelists were not able to tell a difference in tenderness between high or low RFI samples. Fidelis et al. (2017) also reported no significant differences in WBSF between low or high RFI classes, although the increase in mean WBSF with RAC supplementation approached significance ($P = 0.09$) at both day 1 and 8 of post mortem ageing. Gomes et al. (2012) reported that in Nellore cattle there was no evidence that

selection for low RFI caused increased meat toughness. Nascimento et al. (2016) reported that low RFI animals had higher mean shear force for non-aged meat than high RFI animals, but they noted that although it was a statistically significant difference, it was such a small difference that trained panelists would not detect it, and that no differences in shear force values were noted between RFI classes when the samples were aged.

In this thesis, steers that were not selected for RFI exhibited a decrease in LT sensory tenderness rating when they received steroids, whereas this did not occur when steers were from the herd that was selected for low RFI. Why selection for low RFI mitigated the toughening effect of steroids is unclear, but indicates that selection for low RFI may be beneficial in reducing the impact of steroids on meat tenderness. No literature was found that describing studies that explored this interaction but recent research indicates that decreased feed efficiency may be related to increased liver steroid hormone biosynthesis (Novais et al. 2019). De Oliveira et al. (2018) found that RFI was negatively correlated with insulin signalling pathway miRNA modules, a pathway that shares the phosphoinositide 3-kinase (PI3K) pathway with steroid receptors. This suggests that low and high RFI cattle may have differences in steroid hormone receptor chemistry, and this warrants further investigation.

Again in the LT, RFI interacted with steroids, with steers that were not selected for low RFI that did not receive steroids had a higher rating for juiciness than those that either were received steroids or were selected for low RFI but did not receive steroids. These results indicated that either selection for low RFI or use of steroids decreased product juiciness. The reason for this occurring is not clear, as juiciness can arise from increased protein hydration (Honikel and Hamm 1994) or increased intramuscular lipid (Lucherk et al. 2016). Steroid implantation with androgenic steroids such as trenbolone acetate can reduce LT intramuscular fat (Johnson et al. 2013; Smith et al. 2018), which may reduce the sensation of juiciness (Lucherk et al. 2016). Protein hydration is often determined by intramuscular pH, with a high pH increasing the water-holding capacity of beef and moisture of beef (Mahmood et al. 2017). Additional information on intramuscular fat content and pH at cooking is required on the measured samples to be able to deduce causative factors.

In the GM, RFI interacted with steroid use and RAC, with selection for low RFI tending to increase juiciness in all treatments except that of steers that received steroids only, where

juiciness was decreased with selection for low RFI. The supplementation of RAC appeared to mitigate the effect of selection for low RFI on juiciness. Application of RAC appears not to affect intramuscular fat in long-fed cattle (Hunter-Beasley et al. 2018), and so may have preserved sufficient marbling compared to steroids to ensure that meat juiciness was unaffected. However, Basarab et al. (2003) found that RAC supplementation reduced marbling in the LT and Gruber et al. (2008) found that supplementation of beef steers with RAC at 200 mg/head /day did reduce trained sensory panel ratings for juiciness in the LT. The lack of difference observed in the GM due to RAC supplementation in this thesis may have occurred due to the GM being a muscle less likely to marble than the LT (Lee et al. 2017), and therefore spared the effect of reduced juiciness.

There was a three-way interaction between RFI, steroid use and post mortem ageing for juiciness as well, with the day 3 post mortem GM of control steers having a lower mean juiciness rating than that from the same steers that had been aged 12 days. This indicated that sensory juiciness was improved with ageing only in the control steers, suggesting that juiciness in the beef from this population was not related to marbling, and that protein hydration may have changed with ageing in this population of muscles (Honikel and Hamm 1994).

Also in the LT, RFI interacted with steroids, where LT from cattle selected for low RFI had reduced bloody/serumy flavour if they did not receive steroids. In the SM, bloody/serumy flavour was increased in the muscles of steers selected for low RFI with ageing, while that of the control decreased with ageing. Why these interactions occurred is unclear, and no literature was found that examined interactions between RFI and either steroids or post mortem ageing on sensory meat quality. Acheson et al. (2014) found that bloody/serumy flavours were reduced in muscle with increased intramuscular fat, and use of steroids and selection for low RFI have been associated with reduced intramuscular fat (Lucherk et al. 2016; Ahola et al. 2011). Fat-like flavour was reduced with post mortem ageing of SM from control steers, but unaffected in muscles from steers selected for low RFI, suggesting that flavour mechanisms other than those associated with the presence of intramuscular fat may be important in cattle not selected for RFI.

Liver-like flavour was decreased in the SM of high RFI steers with the application of RAC, and was unaffected by RAC in low RFI steers. Liver-like flavour can be associated with heme-iron content of muscles although not always (Meisinger et al. 2006), suggesting that muscle fibre type

may be affected in the SM by RAC and selection for RFI. Cumulatively, the results of this thesis regarding the effects of RFI on meat quality support further research in this area to fully understand the response of the various muscles to selection for RFI and its interaction with production management technologies.

4.5 Purge loss, cooking loss and yield percentage

Steroids had no effect on purge loss, cooking loss and yield percentages of any of the muscles. This result is contrary to Girard et al. (2012) who found that both implanting cattle and supplementation of cattle with RAC increased purge loss. The effect of steroids was however noted by Girard et al. (2012) in the ST muscle, a muscle that was not examined in this thesis. Notably, Girard et al. (2012) found no effect of steroids on purge loss from the GM, but did find that RAC increased purge loss in the GM. In this thesis, treatment with RAC tended to increase the purge loss of the LT, corroborating the findings of Girard et al. (2012) in the GM. Lowe et al. (2014) reported that purge loss during the seven-day commercial display period and loss during cooking were not different between RAC and control fed pigs ($P>0.50$). They also concluded there were no differences ($P \geq 0.44$) between RAC-fed and control-fed pork loins when 14 d post mortem purge loss was evaluated.

Purge loss is an economic concern as it tends to reduce the saleable weight (Offer and Cousins 1992). It can also decrease the juiciness of the meat (Van Oeckel et al. 1999), because purge loss is the water that escapes from the myofibres during ageing and reflects, along with cooking loss, the overall water-holding capacity of meat. Savage et al. (1990) reported that the nutritive value of meat is also affected because soluble protein is lost with purge, therefore, purge losses are not desirable. That Girard et al. (2012) found an effect of treatment on purge loss where none was observed in this thesis may be due to the meat in the Girard et al. (2012) study not being frozen before purge loss was measured. In this thesis, purge loss was measured after the beef was frozen and then thawed, which may have obscured treatment effects on purge loss (Oillic et al. 2011).

Purge loss in this thesis was reduced in GM and SM aged for 12 days post mortem. This was most likely due to the 12 day aged portions of the muscles being aged in one bag and then removed from that bag to be cut into steaks, which were then subsequently re-packaged and frozen until sensory analysis. Purge therefore was ‘lost’ in the transfer from the ageing environment to that of the steak bag. That the LT was not affected by this transfer is unusual, and

perhaps was mitigated by the LT still retaining a subcutaneous fat cap during ageing, which may have mitigated purge loss during that time.

Cooking loss and yield percentages were unaffected by steroid use and RAC in all three muscles. Cooking loss was increased and yield decreased in LT from steers selected for low RFI, while these characteristics in the GM and SM were unaffected. Blank et al. (2017) found that selection for low RFI had no effect on cooking loss of the LT, nor did Nascimento et al. (2016). Why this occurred in this thesis is not clear, but may reflect the effect of freezing, with cooking loss increased with freezing and then thawing (Oillic et al. 2011). If low RFI LT had less fat, then cooking loss would be increased in these samples due to the increased proportion of protein in the steaks.

CHAPTER 5: General Summary

5.1 Significant findings

Substantial work has been done by researchers trying to understand the effects of HGP, BA, RFI and post mortem ageing on meat quality, particularly their effects on tenderness, but little exists on the effects on beef juiciness and other flavour attributes. Interactions between these production factors and their effects on beef sensory qualities are also not described in the literature, and this thesis represents the first foray into this research area. The results of this thesis are also unique in that the effects of the various cattle production management strategies on the sensory attributes are not only examined in the m. *longissimus thoracis*, the most common muscle studied in meat science because it is large, but in other high connective tissue muscles.

This thesis substantiated that the use of anabolic steroids in beef cattle can decrease the tenderness of the LT, GM and SM of a beef carcass even after ageing for up to 12 days. The continuous disparities in results of other researchers who test the effect of steroids on beef tenderness are what make this subject controversial. Collectively, the results from this thesis demonstrated a significant effect of steroids using trained panelist sensory evaluation of the m. *longissimus thoracis*, m. *gluteus medius* and m. *semimembranosus*. The results of this work indicated that the toughening effect of steroids is pervasive as well and occurs not only in low connective tissue muscle like the *longissimus thoracis* but is also evident in the high connective tissue muscles like the *gluteus medius* and the *semimembranosus*.

From the results, it can be concluded that steaks from steers treated with estrogenic and androgenic HGP were the toughest, while steaks from animals that were either treated with RAC or no treatment at all were the most tender regardless of the muscle type. The same can be said for the juiciness of the steak samples, as samples from steers treated with HGP were considered the least juicy by the panelists while steak samples from control steers or those treated with only RAC were the juiciest.

This thesis also substantiated that ageing of beef can increase the tenderness of the cooked product, regardless of muscle type, and indicated that even the tenderness of a muscle considered to have a high level of connective tissue, specifically the SM, can increase with ageing. Muscles

such as the SM are not usually purposefully aged, and this thesis suggests that post mortem ageing of high connective tissue muscles is a viable strategy for increasing product quality by increasing product tenderness. Increasing product tenderness with post mortem ageing was accompanied by limited impact on beef juiciness and flavour regardless of muscle, further supporting the use of post mortem ageing to increase the tenderness and possibly the acceptability of high connective tissue muscles.

This thesis substantiated that RFI can affect the tenderness and flavour of beef by interacting with other production factors. The tenderness of LT from steers selected for low RFI was not affected by steroids and selection for low RFI reduced the bloody/serumy flavour in the same muscle, and could reduce juiciness in the GM when paired with steroid use. The effect of RFI was most evident in interactions, and results of this thesis substantiated that RFI alone had little effect on beef sensory quality in all muscles studied, but may affect beef flavour and cooking loss and yield percentage in the LT.

5.2 Recommended future studies

This thesis did not substantiate that RAC administration decreases the tenderness of beef. The effect of this growth promoting substance is dependent upon its dose, and cattle in this study may not have eaten sufficient RAC to obtain the level of response seen in other studies. Although many studies use 200 mg/head/day, actual feed intake by cattle varies substantially from day to day and week to week, and how much each animal ate may have been less than the target dose. Dose would need to be substantiated by calculating actual RAC intake of each animal, and this is something that future studies should consider doing. That RAC has no effect on beef flavour was substantiated, however, and should reassure the beef industry that use of this growth promoter will not adversely affect this attribute of its product.

Although this thesis indicated that RFI can interact with other production factors to affect beef sensory quality, this thesis is just one study so the interaction effects of RFI will need to be verified in subsequent studies. Because RFI is becoming an important tool for beef producers seeking to increase cattle efficiency without the use of steroids as a response to market pressure to not use steroids, understanding the effects of RFI is important. Further studies on this aspect of

RFI are warranted, given that LT from cattle that do not receive steroids or RAC are most likely destined for international markets where unfrozen storage times are extended to weeks and months, and flavour stability will then become important.

Sustained selection for RFI may increase animal feed efficiency further, which may change bovine muscle metabolism additionally and potential decrease meat eating quality at that point. Future research in this case should address the impact of these generational changes in RFI efficiency on beef eating quality. Although the use of WBSF is suitable for assessing beef toughness, the full sensory experience is best characterized using a trained sensory panel, and beef acceptability best determined by a consumer panel, and incorporation of both of these measures in future studies would provide a complete measure of the quality of Canadian beef.

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Appendices

Appendix A: Panelists recruitment email

I am a graduate student working with Dr. Heather Bruce. I'd like to post an invitation for students and staff of our faculty who enjoying eating beef steaks to our trained sensory panel to taste steaks from Angus cross breed steers.

Could you please help me to spread the recruitment information? Thank you very much and have a good day.

Thanks,

Olalekan Laguda

Study Participants Needed to Characterize Beef Steak!

We are looking for volunteers to participate in a taste study of **beef steak** eating quality from Angus crossbreed steers.

Volunteers will…

- Attend a 1 hour screening process (no compensation)
- Attend 8 sessions of training to become trained sensory evaluation panelists of beef steaks(1 hour each)
- Characterize beef steak samples in 12 sessions (30-minutes each)

All session will be held in AF235@ Ag/For Building

To be eligible: you must…

- Like Beef
- Eat beef steaks often (at least once a week)

You will receive a $200 gift card after completion of the study in recognition of your time and

participation.

For more information or to register for a taste session, please contact Olalekan Laguda (MSc student, Department of Agricultural, Food and Nutritional Science) at the following email address: laguda@ualberta.ca

The plan of the study has been reviewed for its adherence to ethical guidelines and is under review by the Research Ethics Board at the University of Alberta.

Appendix B: Poster for panelist recruitment

Study participants needed to characterize **BEEF STEAKS**

We are looking for volunteers to participate in a trained panel sensory evaluation of beef steaks to characterize their appearance, aroma, texture (tenderness), flavour and juiciness. If you like beef, if you consume beef regularly (at least once a week), then you may enjoy this study. The first activity is a 1 hour screening session (NO compensation for the screening process}. If you qualify after the screening process, there will be 8 training sessions followed by 12 sensory evaluation sessions of the beef steaks. You will need to attend taste panels on Tuesday, Wednesday, Thursday and Friday for three (3) weeks to complete the actual sensory panel for 1 beef muscle.

Compensation will be in the form of a $200 gift card after you successfully complete the 8 training sessions and 12 sensory evaluation sessions.

For more information or registration, please contact Olalekan Laguda, Department of Agricultural, Food and Nutritional Science (AFNS) Via email at laguda@ualberta.ca

The plan for this study has been reviewed for its adherence to ethical guidelines and is under review by the Research Ethics Board at the University of Alberta.MS2_Pro00073730

Beef Tasting Study	Beef Tasting Study	Beef Tasting Study	Beef Tasting Study	Beef Tasting Study	Beef Tasting Study	Beef Tasting Study	Beef Tasting Study	Beef Tasting Study	Beef Tasting Study	Beef Tasting Study

Appendix C: Information and Consent form

Project Information and Consent: Trained sensory panel evaluation of three bovine muscles

Research Investigators:

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Background

You are being asked to participate in a sensory panel to characterize the eating quality of beef steak because you have indicated that you like and regularly consume beef steak. The cattle from which the beef for this study was obtained were treated with steroids and/or ractopamine hydrochloride (which are growth promoting substances that are approved for use by the Canadian Food Inspection Agency and routinely used in Canadian cattle production) or were not treated and served as controls. Steroids used in the cattle from which the beef in this study was obtained were prescribed by a veterinarian, as was the ractopamine hydrochloride, which is available commercially as Optaflexx™. Steroids and ractopamine are used in cattle to increase lean muscle yield. The effects of these treatments on meat tenderness are not well understood and may change the eating characteristics of the beef, thus there is a need to use a sensory panel to characterize the eating quality of beef from these production systems.

Purpose

The intent of this project is to characterize the eating quality of beef steaks from crossbred Angus steers raised at the Roy Berg Kinsella Research ranch by generating trained sensory panel

descriptions of the flavour and texture characteristics of the steaks. The results of this study will be used in support of a master's degree thesis and to produce a project report for the funding agency.

Sensory Study Procedures

If you would like to participate in this study, we will invite you to a one-hour screening session to determine if you have 'above average' taste, smell and texture food characterization capabilities. In the screening session we will ask you to identify the basic tastes in water solutions, rank three food samples in order of their tenderness, and describe the taste, smell and texture of two cooked beef samples. We will also ask you to complete a questionnaire.

If you pass the screening process, we will invite you to participate in the trained sensory panel to characterize the beef steaks. You will be asked to attend 8 one-hour training sessions with the other trained sensory panel members. During panel training you will learn to characterize the flavour and texture characteristics of the steaks. These sessions will take place four days per week; from Tuesday to Friday at a time convenient for all panel members. You will then evaluate steaks from semimembranosus (SM) muscle in 12 sessions over 3 weeks. The evaluation sessions will take about 30 minutes and will again take place five days per week; from Monday to Friday at a time convenient for all panel members.The sensory panel will take place in AF building 2-35.

Benefits

There will not be any direct benefits to you for participating in this study. The results from this study will help inform the principal investigator and study sponsor about the eating quality characteristics of the production systems describe in the background.

Incentives

If you successfully complete the 8 training sessions and the 12 evaluation sessions of the trained sensory panels (for one muscle) you will receive a $200 gift card in acknowledgement of your time and contribution to the study. If you withdraw before the end of the study, the incentive will be pro-rated based on the number of sessions you attend.

Potential Risks

There are no risks other than those everyday risks associated with consuming beef, water, unsalted crackers, cheddar cheese and tofu. The cattle were raised at the University of Alberta's Roy Berg Kinsella Research ranch, and slaughtered at Love's Meat in Vegreville, which is a provincially inspected facility. The meat samples will be prepared under safe food conditions, internal cooking temperature will be monitored using a thermocouple and cooked samples will be kept in a heated oven to prevent microbial growth pending evaluation. All other food products will be purchased from a grocery store. If you have any allergies, sensitivities or intolerances to food used in the study, you should not participate in this study.

Voluntary Participation and Withdrawal from the Study

Participation in this study is completely voluntary and you are under no obligation to participate. Even after you have agreed to participate in the sensory panel, you may withdraw from the study at any time. If you withdraw, we will continue to use your data unless you ask us not to do so. After the sensory panel is completed, we will not be able to withdraw your data as we will destroy the participant list and your data will become anonymous.

Confidentiality

Anonymity cannot be guaranteed in the sensory panels as several people will participate at the same time and will be visible to one another. A participant number will be assigned to link evaluations from all sessions. We will have a list with participant names and numbers as a reference should participants forget their number. Also, we will record email addresses in the list so that we may send an attendance reminder before each evaluation session. When the study ends, the list will be destroyed. Participants will not be personally identified in the results of this study; we present our results in aggregate form. All study documents will be kept on file in a locked room in a locked cabinet at the University of Alberta for a minimum of 5 years. Computer files will be encrypted. The final result and statistical data of this project may be reported in scientific journal publication by the study team. Individual responses will be kept confidentially. We may use the data from this study in future research, but if we do this it will have to be approved by a Research Ethics Board.

The food products and ingredients of some food products in this study are listed below. Do you have any allergies, intolerances, or sensitivities to any of the following food or ingredients?

Distilled water
Unsalted cracker: Enriched Wheat Flour, Soybean Oil, Baking Soda, Salt, Malted Barley Flour, Yeast Amylase Protease, Sour Dough Culture. Contains: Wheat, Barley
Beef steak
Cheddar cheese
Tofu

If you have answered "yes", please stop and tell us immediately.

Consent Statement

I have read this form and the research study has been explained to me. I have been given the opportunity to ask questions and my questions have been answered. If I have additional questions, I have been told whom to contact. I agree to participate in the research study described above and will receive a copy of this consent form. I will receive a copy of this consent form after I sign it.

_____ _____

Participant's Name (printed) and Signature Date

_____ _____

Name (printed) and Signature of Person Obtaining Consent Date

Appendix D: Panelist screening Questionnaire

Contact information

Name: _____

Phone number: _____

Email address: _____

Availability:

1. Are there any weekdays (Monday-Friday) that you will not be available from 17-september-2018 – 30-november-2018?

What time is most convenient for you?

Pick all that applies;

a. 10 – 11 am
b. 10:30 – 11:30 am
c. 2- 3 pm
d. 2:30-3:30 pm

Health:

1 Do you have any of the following?

Dentures

Diabetes

Oral or gum disease

Hypoglycemia

Food allergies

Hypertension

Thyroid condition

Pregnant

2 Do you take any medications which affect your senses, especially taste and smell?

For training product purposes, are there any food products you DO NOT eat?

Food Habits:

1. Are you currently on a restricted diet? If yes, please explain.
2. What is (are) your favourite foods?
3. What is (are) your least favourite foods?
4. What foods do you not eat because of insensitivities, intolerances, allergies or dislikes?

Insensitivities ----------

Intolerances----------

Allergies----------

1. How would you rate your ability to distinguish smells and tastes?

	Smell	Taste
Better than average	_____	_____
Average	_____	_____
Worse than average	_____	_____

FLAVOUR QUIZ:

1. What are some other foods that taste like yoghurt?

---

2. What would you say is the difference between flavour and aroma?

---

3. What would you say is the difference between flavour and texture?

DESCRIPTION OF BEEF ATTRIBUTES

Briefly in your own words explain what you understand by the following in relation to beef

Initial Tenderness

Beef flavour

Juiciness

Overall Tenderness

IDENTIFICATION OF BASIC TASTES IN WATER:

There are five samples that contain substances that represent the five basic tastes (sweet, sour, salty, bitter, umami) plus one water sample for a total of six samples. They have been labelled with three-digit codes.

Taste the samples in any order and identify the basic taste it represents. Please rinse your mouth with water between each sample.

Solution	Basic taste Identified
774	
811	
634	
253	
907	
621	

TENDERNESS RANKING:

You have been presented with 2 samples, rank each sample according to its tenderness.

0= least tender **15= most tender**

A **B**

_____ _____

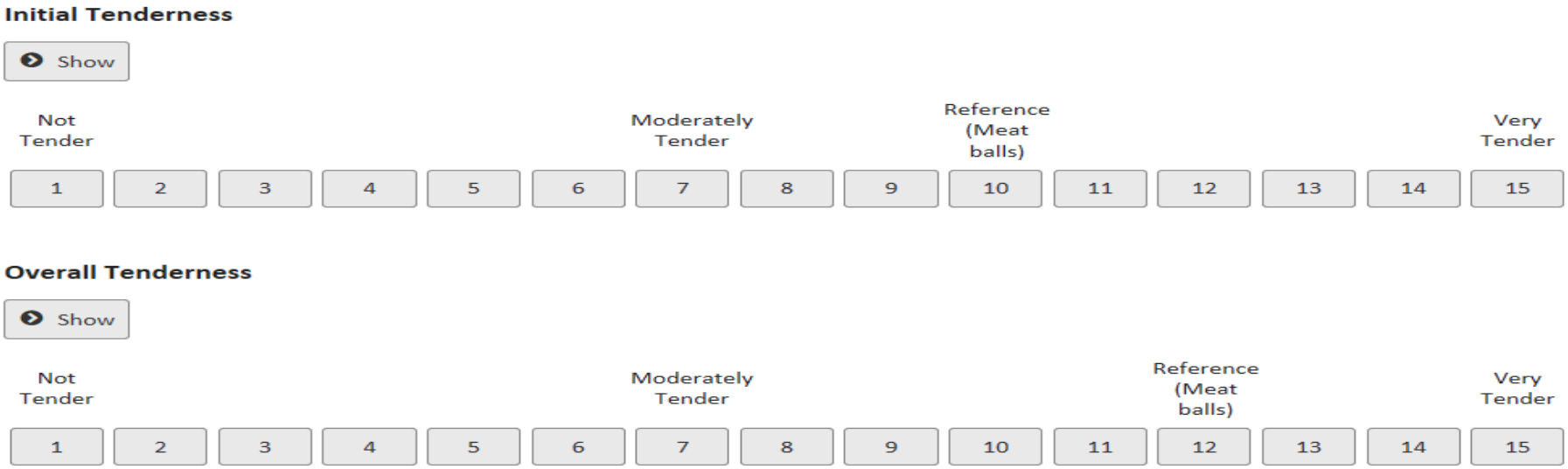
Which of the sample is tougher?

Appendix E: Definition sheet and reference standards for sensory attributes to be evaluated

ATTRIBUTES	END POINTS	DEFINITION	REFERENCE STANDARDS
INITIAL TENDERNESS	1 – 15 Not Tender- Very Tender	Initial force used to bite sample using front teeth from top down with the grain. Bite through centre of the meat balls.	MEAT BALLS=10
OVERALL TENDERNESS	1 – 15 Not tender-Very tender	Total force used to chew sample multiple times before swallowing.	MEAT BALLS=12
JUICINESS	1 – 15 Not juicy- Very juicy	Amount of perceived juiciness / moisture during chewing.	BEEF STEAK=8
BEEF FLAVOUR IDENTITY	1 – 15 Weak Beef flavour-Strong Beef flavour	Aroma and flavour generally associated with beef flavour in the sample.	BEEF BROTH=5, GROUND BEEF=7, SPICED GROUND BEEF=11, BEEF STEAK=8.
BROWN/ ROASTED	1 – 15 Weak brown roasted – Strong brown roasted	Aroma and flavour generally associated with beef that has been broiled.	GROUND BEEF=8, BEEF STEAK=8
BLOODY/ SERUMY	1 – 15 Weak bloody serumy – Strong Bloody serumy	Aroma and flavour generally associated with blood on undercooked or rare meat.	GROUND BEEF=2
FAT-LIKE	1 – 15 Weak Fat-like flavour–	Aroma and flavour generally associated with cooked animal fat.	

	Strong fat-like flavour		
LIVER-LIKE	1 – 15 Weak liver like – Strong liver like	Aroma and flavour generally associated with cooked meat liver.	BEEF LIVER= 12-14
SULPHUR LIKE/OFF-FLAVOUR	1 – 15 Weak sulphur/off flavour – Strong sulphur/off flavour	Aroma and flavour generally associated with sulphur like/off flavour in meat.	GROUND BEEF=1
AFTER TASTE	1 – 15 Weak aftertaste – Strong aftertaste	Strength of the beef taste in the mouth after swallowing.	BEEF STEAK=5

Appendix F: Panelists sensory evaluation questionnaire



Juiciness

Show

Not Juicy						Moderately Juicy	Reference (Beef steak)							Very Juicy
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

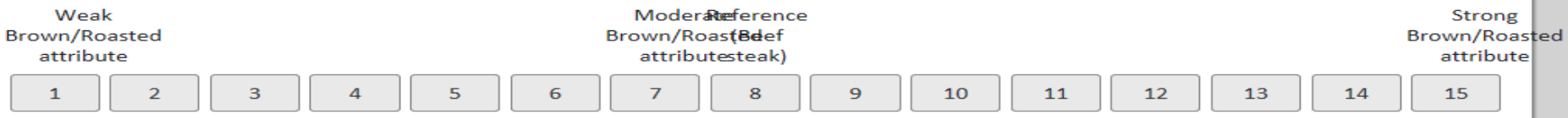
Beef Flavour Identity

Show

Weak Brown/Roasted attribute						Moderate Brown/Roasted attribute	Reference (Beef steak)							Strong Brown/Roasted attribute
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

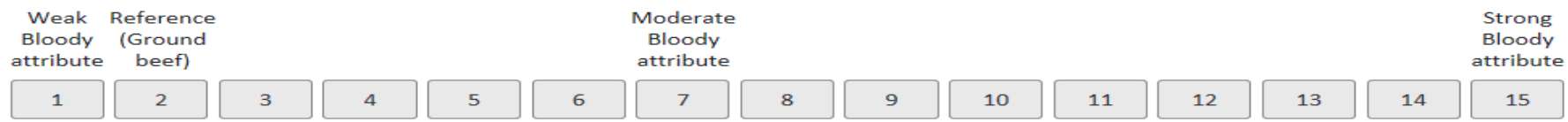
Brown / Roasted

Show



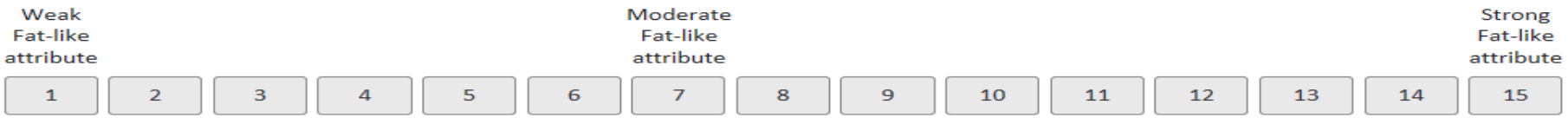
Bloody / Serumy

Show



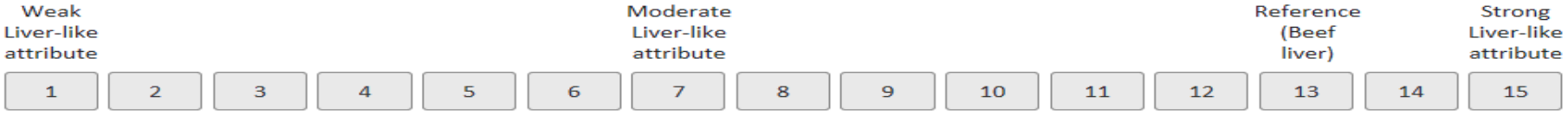
Fat-like

Show



Liver-like

Show



Sulphur-like/Off flavour

Show

Weak Putrid attribute (Reference)						Moderate Putrid attribute								Strong Putrid attribute
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Aftertaste

Show

Weak Aftertaste				Reference (Beef steak)		Moderate Aftertaste								Strong Aftertaste
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Appendix G: SOP for sensory project

EX-017.1 Trained Panelists Evaluation of Three Bovine Muscles

1.0 DESCRIPTION

1.1 This document describes the methods used for conducting a free choice profiling sensory panel assessing the aroma, flavour, juiciness, texture, and tenderness of beef steaks treated with steroids and or ractopamine.

2.0 RESPONSIBILITY

2.1 Personnel tasked with ensuring this study is done accurately and correctly in the food sensory laboratory.

2.2 Personnel with management and oversight responsibilities regarding sensory panel performed in the food sensory laboratory.

3.0 HAZARD ASSESSMENT AND SAFETY

3.1 Cooking surfaces will be very hot.

3.2 Cooked steaks will be served. All surfaces and equipment must be properly cleaned and sanitized.

3.3 Good food handling practices must be adhered to.

4.0 EQUIPMENT / MATERIALS

1.1 Clamp shell

1.2 Tong

1.3 Foam bowls

1.4 Foam bowl lids

1.5 cutleries

1.6 $50 gift cards

1.7 Paper towel

1.8 Cracker biscuits

1.9 Treats (chocolates & sweets)

1.10 pencils

1.11 Drinking glass

1.12 Water jug

1.13 Trays

1.14 Pencil sharpener

1.15 Erasers

1.16 Ruler

1.17 Thermocouple

1.18 Hairnets

1.19 Laboratory coat

1.20 Oven

1.21 Glassware

1.22 Sensory booth

1.23 Forms (questionnaires, consent form, demographic survey form)

1.24 Markers

1.25 Cutting boards

5.0 PROCEDURES

5.1 Recruiting panelists

5.1.1 Panel requires panelists to be available for the duration of the study. Posters must be placed to attract panelists a minimum of three weeks before panel is set to run.

5.1.2 Compose a standard email as a reply to all interested panelists. Include a copy of the consent form and the information sheet. This will inform panelist of any potential allergy risk and responsibilities. Be specific regarding the time and location to avoid confusion.

5.2 Preparation for personnel

5.2.1 All personnel who will be in contact with the panelists are required to avoid the use of perfumes and make-up products to avoid confusing the sensory apparatus of the panelists.

5.2.2 To present the best possible image, all personnel must ensure to wear clean laboratory coats, hairnets and no jewelry.

5.3 preparation of product

5.3.1 Defrost meat for 24 hours at 4^{o}C.

5.3.2 Turn on the clam shell and set the temperature to 350F

5.3.3 Remove meat from vacuum sealed bag and pat it down with paper towel. Keep label close and keep track of the label so meat samples doesn't get mixed up.

5.3.4 Insert thermocouple in the middle of the meat sample to monitor the internal temperature.

5.3.5 Place meat on clam shell and cook until the internal temperature of the meat is 71°C.

5.3.6 Remove cooked meat from clam shell and place on a cutting board.

5.3.7 Place cooked meat in glassware and put in heated oven.

5.3.8 Cut of all edges of meat and dispose; but leave the bottom and top crust intact. Look at the meat; you want to cut strips of meat across the shorter distance. Use a ruler to mark 0.5cm widths on the meat, and then cut even strips of meat using a knife.

5.3.9 Place the cubes of meat in labelled foam bowl.

5.4 Serving meat sample

5.4.1 Fill water jug and cover with cling film and keep till when needed.

5.4.2 Set up a tray for each participant. Each tray should have a napkin placed at the top with sharpened pencil, 3 cracker biscuit and a glass filled with room temperature water.

5.4.3 Assign each sample a 3-digit code. Prepare in advance who will get each sample. Each panelist will taste the 8 different types of treatments available.

5.4.4 When serving a sample, place two cubes of meat in a foam bowl and cover with a foam lid and label it. Do this only when the panelist is ready, or the meat will become cold.

5.4.5 Fill the glass with room temperature water. Place the first four samples on the pre-set tray, along with three sets of knives and forks, after the panelist are done the reaming four samples will be served to the same set of panelists. Questionnaires must be on the tray prior to serving the panelist.

5.4.6 Serve samples to panelist.

5.4.7 Collect trays and questionnaire.

5.4.8 All food waste and disposable bowls including cutleries should be thrown in the garbage and garbage bag should be put in “land fill” at the end of the day.

5.4.9 Give a $50 gift card after the panelist has completed all evaluations as an appreciation of their time.

6.0 REFERENCES

6.1 Lawson, J.2010.Design and analysis of experiments by SAS. Taylor and Francis group, FL, USA. P.255-259.

7.0 CHANGE CONTROL

Version		Date
	This is the first version of a new document	

8.0 SIGNATURES

8.1 Primary author: Olalekan Laguda, Graduate student

Signature	Date

8.2 Reviewer: Dr. Heather Bruce, Principal Investigator

Signature	Date

*** Please refer to the sensory plan document provided for the outlined schedule of which samples will be served and what time it will be served***

1st Kill date	Study Animal ID	Treatment			Sensory 3-digit codes for GM samples	Sensory 3-digit codes for LT samples	Sensory 3-digit codes for SM samples
28-Sep	45C	RFIE	STE	RACY	334	192	020
	235C	RFIE	STE	RACN	918	576	287
	383C	RFIC	STE	RACN	962	497	082
	245C	RFIE	STE	RACN	998	267	941
	249C	RFIE	STE	RACY	203	959	331
	267C	RFIC	STE	RACN	008	521	476

	349C	RFIC	STEN	RACY	733	602	655
	367C	RFIC	STEY	RACY	750	599	917

2nd Kill date	Study Animal ID	Treatment			Sensory 3 digit codes for GM samples	Sensory 3 digit codes for LT samples	Sensory 3 digit codes for SM samples
05-Oct	115C	RFIE	STEY	RACY	980	130	603
	153C	RFIE	STEN	RACY	976	734	103
	207C	RFIE	STEY	RACN	731	002	904
	221C	RFIE	STEN	RACN	163	768	245
	318C	RFIC	STEN	RACY	748	186	694
	435C	RFIC	STEY	RACN	672	284	235

	441C	RFI	STE	RAC	344	641	975
		C	Y	Y			
	447C	RFI	STE	RAC	365	419	299
		C	N	N			

3rd Kill date	Study Animal ID	Treatment			Sensory 3 digit codes for GM samples	Sensory 3 digit codes for LT samples	Sensory 3 digit codes for SM samples
12-Oct	27C	RFI	STE	RAC	445	588	358
		E	Y	N			
	81C	RFI	STE	RAC	318	631	006
		E	Y	Y			
	219C	RFI	STE	RAC	134	572	926
		E	N	N			
	237C	RFI	STE	RAC	788	590	912
		E	N	Y			
	271C	RFI	STE	RAC	841	121	260
		C	Y	N			
	297C	RFI	STE	RAC	568	201	914
		C	N	Y			
	345C	RFI	STE	RAC	019	831	083
		C	Y	Y			
	427C	RFI	STE	RAC	234	661	653
		C	N	N			

4th Kill date	Study Animal ID	Treatment			Sensory 3 digit codes for GM samples	Sensory 3 digit codes for LT samples	Sensory 3 digit codes for SM samples
19-Oct	65C	RFI	STE	RAC	587	555	915
		E	N	N			
	67C	RFI	STE	RAC	307	880	728
		E	N	Y			
	177C	RFI	STE	RAC	985	811	028
		E	Y	N			
	341C	RFI	STE	RAC	755	418	059
		C	N	Y			
	347C	RFI	STE	RAC	054	732	913
		C	Y	N			
	369C	RFI	STE	RAC	440	702	839
		C	Y	Y			
	443C	RFI	STE	RAC	791	886	628
		C	N	N			

5th Kill date	Study Animal ID	Treatment			Sensory 3 digit codes for GM samples	Sensory 3 digit codes for LT samples	Sensory 3 digit codes for SM samples
26-Oct	13C	RFI	STE	RAC	842	554	795
		E	Y	N			
	39C	RFI	STE	RAC	916	195	232
		E	N	Y			
	159C	RFI	STE	RAC	392	700	451
		E	Y	Y			
	187C	RFI	STE	RAC	790	233	495
		E	N	N			
	327C	RFI	STE	RAC	060	170	206
		C	Y	N			
	355C	RFI	STE	RAC	644	147	154
		C	Y	Y			
	439C	RFI	STE	RAC	153	119	271
		C	N	Y			
	445C	RFI	STE	RAC	364	709	970
		C	N	N			

6th Kill date	Study Animal ID	Treatment			Sensory 3 digit codes for GM samples	Sensory 3 digit codes for LT samples	Sensory 3 digit codes for SM samples
07-Nov	73C	RFI	STE	RAC	903	840	258
		E	N	Y			
	85C	RFI	STE	RAC	875	398	483
		E	Y	Y			
	107C	RFI	STE	RAC	536	938	056
		E	N	N			
	203C	RFI	STE	RAC	817	649	239
		E	Y	Y			
	205C	RFI	STE	RAC	911	657	444
		E	Y	N			
	343C	RFI	STE	RAC	776	484	697
		C	N	N			
	247C	RFI	STE	RAC	531	126	832
		E	N	Y			
	231C	RFI	STE	RAC	835	139	796
		E	Y	N			

Each Animal ID has been assigned 3-digit codes for each muscle cut. The different treatments are represented in several kill dates. For example, one panelist will evaluate all the 8 GM samples with different combinations of treatments.

RFI	STE	RAC	Treatment Number assigned for Latin square
E	Y	Y	1
RFI	STE	RAC	
E	N	N	2
RFI	STE	RAC	
C	N	N	3
RFI	STE	RAC	
E	Y	N	4
RFI	STE	RAC	
E	N	Y	5
RFI	STE	RAC	
C	Y	N	6
RFI	STE	RAC	
C	N	Y	7
RFI	STE	RAC	
C	Y	Y	8

Sample presentation order								
Panelist	1	2	3	4	5	6	7	8
1	1	2	8	3	7	4	6	5
2	2	3	1	4	8	5	7	6
3	3	4	2	5	1	6	8	7
4	4	5	3	6	2	7	1	8
5	5	6	4	7	3	8	2	1
6	6	7	5	8	4	1	3	2
7	7	8	6	1	5	2	4	3
8	8	1	7	2	6	3	5	4

RFI= Residual Feed Intake.

STE= Steroids.

RAC= Ractopamine.

N= No.

Y= Yes.

E= Efficient.

C= Control.

Appendix H: Sample Cooking Method
Clam Shell

Clam shell procedures

• Plug in the grill and allow preheating at least 10 minutes or according to manufacturer's instructions.
• Record sample weights.
• Insert thermocouples into the geometric center of the steaks, chops, or patties as described above. Record initial temperatures.
• Place the meat on the grill surface so that the thermocouples are accessible with the lid closed. Close the lid. Grill temperatures may vary and should be measured and reported in the experimental section of the manuscript.
• Steaks and should not need to be turned during grilling because they are heated from top and bottom.
• Remove the meat when it reaches the desired internal temperature (71°C is the standard for cuts of all species). Record cooking time.
• Immediately record cooked weights for determination of cooking losses.
• Characterize steaks as soon as possible.

• Fat and ends (bones and epimysia connective tissue) from steaks were removed before cutting into 2 cm cubes, and then wrap all cubes with foil and put in a heated oven preheated at 80°C (Huidobro FR, 2001) before serving.
• Before serving, take out two cubes and put them into a plastic container (with 3-digit code). And 3-digit code should be placed on the containers 24h prior to use.
• Each panelist should rinse their mouth between samples with unsalted crackers (one piece), and room temperature distilled water (approximate 30ml)
• A total 8 samples will be evaluated in a panel session for each panelist. The presentation order should follow the Williams' Latin Squire to reduce bias related to serving position and carryout effects.

Measures	
WT meat + drip + package	
WT package	
WT plate	
WT plate + meat (no drip)	
Temp in	
Cook time in	
Temp end	
Cook time end	
WT plate	
WT plate + meat (no cooking drip)	
WT cook meat	