THESIS

EFFECTS OF LUBABEGRON SUPPLEMENTATION ON CARCASS TRAITS, MUSCLE FIBER TYPE, PROTEOME PROFILE AND MEAT QUALITY ATTRIBUTES OF FINISHED FEEDLOT STEERS

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ABSTRACT

EFFECTS OF LUBABEGRON SUPPLEMENTATION ON CARCASS TRAITS, MUSCLE FIBER TYPE, PROTEOME PROFILE AND MEAT QUALITY ATTRIBUTES OF FINISHED FEEDLOT STEERS

Two thousand one hundred and sixty (2,160) British and Continental crossbred steers were supplemented (1, 4, 3.2 or 5.0 g/ton (DM basis) Lubabegron and a control diet (Experior®; EX, Elanco Animal Health) for the last 28, 56, or 84 d of the finishing period resulting in twelve treatment combinations. Fifteen pens (12 hd/pen) were allocated to each treatment combination consisting of a dose and feeding duration. A total of five harvest cycles were conducted, consisting of 432 head per cycle. Each harvest cycle consisted of 3 blocks, each block contained all dosages and each block was associated with a specific feeding duration.

Hot carcass weights (HCW), marbling scores (MS), adjusted fat thickness (aFT), *longissimus* muscle area (LMA), kidney pelvic and heart fat percentage (KPH), and USDA calculated yield grade (YG) were evaluated for all carcasses (N = 2160). No dose x feeding duration (FD) interaction ($P > 0.05$) was present for any of the characteristics measured. Supplemented cattle produced heavier ($P < 0.05$) carcass weights, larger ($P < 0.05$) LMAs and decreased ($P < 0.05$) YGs. As feeding duration was extended from 28 to 56 and 84 d, carcass weights were increased ($P < 0.05$). Control cattle produced MS that were significantly higher than those that were supplemented EX at the highest dose; nonetheless MS remained within USDA Premium Choice (MT00-99). Whereas, EX supplementation did not affect aFT and KPH.

A subset of carcasses (N= 540) (3 carcasses/pen) that graded USDA Low Choice (SM00-99) were selected for the purpose of objective color, muscle fiber typing, proteome analysis, and the evaluation of the effect of postmortem aging on tenderness and palatability during harvest. As dose increased ($P < 0.05$) to 3.2 and 5.0 g/ton steaks became less ($P < 0.05$) red (a*), less ($P < 0.05$) yellow (b*), and less ($P < 0.05$) saturated than the controls. Striploin steaks collected during fabrication (before aging) were analyzed for muscle fiber typing (N = 96, n = 8). No detrimental shifts ($P > 0.05$) were observed for muscle fiber type as it relates to meat quality. The muscle fiber type IIX cross sectional area remained similar across the majority of treatment groups, except for decrease in CSA seen in cattle fed 5.0 g/ton for the final 56 and 84 d of feed.

Meat quality attributes were measured using trained sensory panels, slice shear force (SSF) and Warner-Bratlzer shear force (WBSF). Striploins from the right side of each carcass were collected, fabricated into 2.54-cm steaks, and aged for 0, 7, 14, 21, and 28 d postmortem. Steaks for all postmortem aging periods were evaluated using SSF and WBSF, whereas, only those aged for 14 d were evaluated by trained panelists. Non- supplemented cattle produced striploin steaks that were juicier and more tender ($P < 0.05$) than those from EX supplemented cattle regardless of dose, and no differences ($P > 0.05$) were observed as a consequence of FD. All steaks (supplemented and non-supplemented) subjected to a minimum 7 d of PM aging produced WBSF that were less than 3.9 kg, and therefore eligible to be labeled as "Certified Very Tender." Once 21 d of postmortem aging was reached, no differences ($P > 0.05$) in tenderness were observed between the treatments.

Based on meat quality attributes, six samples each (N = 24, n = 6) from four treatments (control, low dose for 28 days, high dose for 28 days, and high dose for 84 days) were selected for proteome analysis using a chemical labelling approach know as tandem mass tag (TMT). Experior

supplementation influenced expression of proteins involved in muscle contraction, calcium signaling, transport, growth factor, and proteasome activation. Myosin light chain 3 (MYL3) was associated with an improved tenderness and carcass grading, which could be reflective of the increased intramuscular fat content. The proteins identified such as hemoglobin subunit α (HBA), hemoglobin subunit β (HBB), and alpha-1-acid glycoprotein (ORM1) were suggestive of increased vascularization in muscles as a response to EX supplementation.

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CHAPTER 1 - REVIEW OF LITERATURE

1.1 Introduction

For decades growth enhancing technologies they have been an integral part of the livestock feeding systems in both cattle and hog production for their impact on growth performance, efficiency and profitability. As the world population continues to increase, improving the productivity of beef cattle while maintaining eating quality has become a fundamental need for the livestock industry.

Beef tenderness is arguably one of the most important traits affecting beef palatability (Dikeman, 1987; Savell et al., 1987; Miller et al., 1995; Miller et al., 2001). Due to the utilization of growth-promoting technologies in today's production systems, the postmortem management of sub-primal cuts becomes vital as producers begin to take a more aggressive approach to improve cattle performance (Garmyn and Miller, 2014). Therefore, the production of tender beef products must be addressed from the perspective of both ante and postmortem management (Arp, 2012).

The current state of livestock production and the economy has dictated an increase in the use of pharmacological agents to make beef and pork production more efficient and affordable (Arp, 2012). Over the past decade, an alternative class of growth promotants known as β-adrenergic agonists (βAA) have thrived within the livestock industry due to the drastic increase in performance and carcass yields (Garmyn and Miller, 2014). Beta-adrenergic agonists, otherwise known as repartitioning agents, are technologies that producers have continued to utilize to increase productivity gains, efficiency, and overall production yields. Beta-adrenergic agonists are members of a large family known as G protein-coupled receptors, which play a significant role in

the regulation of energy distribution (Chikuni et al., 2008). For example, βAA are most notably recognized for increasing the accretion of skeletal muscle while simultaneously diminishing fat deposition (Bell et al., 1998; Mersmann, 1998), resulting in the maximization of lean meat yields seen by the beef industry.

Two major βAA compounds exist in the market for use in food animals in the United States, namely, Ractopamine hydrochloride and Zilpaterol hydrochloride (Dilger, 2015). Ractopamine hydrochloride has been widely used within hog production since the approval in 1999 and was later approved in 2003 for cattle (Elanco Animal Health) along with Zilpaterol hydrochloride (Merck Animal Health) in 2006. The approval of βAA's has allowed cattle feeders to combine the growth-promoting effects of steroidal implants with repartitioning agents (Arp, 2012). With increased growth and performance comes increased skeletal muscle accretion and improve overall carcass yield (Perry et al., 1991; Kellermeier et al., 2009; Parr et al., 2011). Over the past several decades, cattle management practices have changed to reflect the changing demands of the consumer (Apple et al., 1991). Unfortunately, adverse effects in carcass quality, shear force values, and eating quality have been reported and are heavily dependent on the aggressiveness of the production system (Garmyn and Miller, 2014).

As market incentives continue to surge, the need for beef producers to increase growth rates and reduce costs of live weight gain have become unavoidable (Roeber et al., 2000). In response, the U.S. cattle industry rapidly adopted the use of growth-promoting implants as routine management practices. For decades, cattle producers have primarily utilized estrogenic and androgenic implants to enhance live animal growth in fed cattle. For over 50 years, nearly 97% of livestock within the feeding industry have received hormonal implants; where 77% were implanted once, and 30% were implanted twice (Adeola et al., 1992; Duckett and Andrae, 2001) in order to

increase animal growth and profitability in fed cattle (Barham et al., 2003; Tatum, 2006). The growth implants increase average daily gain (ADG), improve feed efficiency, and produce a leaner, more muscular carcass, while reducing the number of days cattle are on feed (Apple et al., 1991; Foutz et al., 1997). The majority of implants on the market are considered estrogenic, androgenic, or a combination of the two. Woerner and others (2011) documented roles and contrasted the initial and terminal hormonal implant regimens.

Use of either Ractopamine hydrochloride (RAC) or Zilpaterol hydrochloride (ZH) have been documented to elicit improvements in live weight gain (LWG), ADG, and feed efficiency similar to hormone implants (Arp, 2012). Moreover, these compounds have shown overwhelming effects on HCW, longissimus muscle area, and carcass cutability (Gruber et al., 2007; Rathmann et al., 2009; Shook et al., 2009; Vogel et al., 2009; Hilton et al., 2010; Scramlin et al., 2010). Simultaneously, research has reported the negative effect of these compounds on carcass quality traits (Reiling and Johnson, 2003), product tenderness (Schneider et al., 2007), marbling scores, incidence of dark cutters (Roeber et al., 2000), and consumer taste panel scores (Barham et al., 2003). It has been documented for both Ractopamine (Scramlin et al., 2010; Woerner et al., 2011; Boler et al., 2012) and Zilpaterol (Avendaño-Reyes et al., 2006; Garmyn et al., 2010; Scramlin et al., 2010) that supplemented cattle are less tender (Consumer Union, 2013). However, evidence from Scramlin et al. (2010) and Boler et al. (2012) indicated that the decrease in tenderness associated with Ractopamine could be overcome with postmortem aging.

Apprehensions regarding animal mobility and lameness led to announcements from major packers such as Cargill in 2013 and Tyson Foods, Inc., in 2014 that they would stop accepting Zilmax-fed beef. Soon after such claims were publicized, Merck, the manufacturer of Zilmax, withdrew Zilpaterol from U.S. and Canadian markets (Centner et al., 2014). Additionally,

Welshans (2019) reported that JBS USA eliminated use of ractopamine from their pork production system in August 2019 along with Smithfield Foods as several countries have a zero tolerance policy for ractopamine usage in pork, which was impacting the ability of meat processors to export.

1.2 Hormone Implants

1.2.1 History

Conventional beef production systems in the U.S. have involved extensive use of anabolic implants during one or more phases of production for over 50 years (Barham et al., 2003; Tatum, 2006). To maintain the cost-effectiveness and sustainability of the beef industry, the ability to manage livestock to meet consumer demands is necessary (McPhee et al., 2006). Currently, 92.3 percent of all feedlot cattle are implanted at least one time during the finishing phase (Reuter et al., 2017). Both steers and heifers entering conventional feedlot systems receive two sequential hormone implants throughout the finishing period. The initial implant is given at processing immediately after arrival, and the second is given approximately 70 to 120 d before harvest (Nichols et al., 2005).

There are two basic categories of compounds used in hormonal implants, estrogenic (estrogen) and androgenic (testosterone) compounds. Implantation with estrogenic and androgenic hormones improves the overall efficiency of feedlot cattle by increasing final body weight and decreasing days on feed (Perry et al., 1991). In addition, hormonal implants increase net returns by increasing both the rate and efficiency of weight gain by enhancing protein accretion (Tatum, 2006). The first “steroid-like” hormone used in beef cattle for growth, efficiency, and lean meat promotion was diethylstilbestrol (DES) in 1954. However, DES was banned in 1979 due to its’ potential carcinogenic effects (Preston, 1999). Following the approval of DES, Zeranol and

Trenbolone acetate (TBA) implants were approved for use in cattle in 1969 and 1987, respectively (Preston, 1999). Currently, there are 26 different anabolic compounds that exists among the three major producing companies; Elanco®, Merck® and Zoetis®.

According to Smith et al. (2005) "reduced quality grade and tenderness due to implants" ranked top as the "top 10 quality challenges" that are facing the United States beef industry. Woerner et al. (2011) stated that understanding of the combined effects of implanting strategies and the supplementtion of βAA is vital in order to improve cattle performance without negatively affecting beef quality.

1.2.2 Mechanism of Action

The actions of androgens and estrogens may be different with androgens having a direct effect on the muscle cell. In contrast, estrogens may act indirectly through the regulation of growth hormone and insulin in the plasma (Heitzman, 1979).

Despite widespread use, relatively little is known about the biological mechanism by which androgenic and estrogenic steroids enhance the rate and efficiency of muscle growth in cattle (Dayton and White, 2013). Estrogenic hormones are believed to act on the anterior pituitary to increase circulating levels of somatotropin and growth hormone (GH; Trenkle, 1997). Circulating GH binds specific GH receptors on the liver to increase the production of insulin-like growth factor-I (IGF-I), which is transported by IGF-I binding proteins to tissues and acts to increase protein accretion and stimulate bone growth (Hossner, 2005). Implanting cattle with estrogenic compounds results in an increase in the number of GH receptors on the liver (Breier et al., 1988a), which yield higher levels of circulating IGF-1 (Breier et al., 1988b). Insulin-like growth factor

then binds to a specific IGF-receptor which activates the pathway involving PI3-Kinase which directly leads to the increase of the accretion of protein (Arp, 2012).

These steroids may modify either the pituitary or the hypothalamus, making the pituitary more responsive to GHRH and thereby causing higher secretion of GH (Trenkle, 1997). According to Johnson et al. (1998) the increase of IGF-I increases the proliferation of cells which serves to increase muscle hypertrophy.

Androgenic hormones work directly on their specific receptors on the muscle cell (Heitzman, 1979). As Wu (1997) reported, the mode of action regarding androgens in not fully understood; consequently, it is assumed that they elicit a similar response as a estrogenic through intracellular signaling, while also blocking the potential catabolic effects of glucocorticoid hormones cascade that stimulates protein accretion (Wu, 1997). Additionally, androgens have been reported to regulate protein synthesis and degradation, resulting in increased protein accretion and decreased rate of protein turnover (Vernon and Buttery, 1976).

Levels of IGF-1 were found to be amplified when estrogens were administered in combination with androgens such as Trenbolone acetate (TBA) (Johnson et al., 1996; Johnson et al., 1998). It is because of these independent mechanisms by which estrogens and androgens impart their physiological effects that estradiol and TBA are used in combination to produce additive anabolic affects in implanted cattle (Trenkle, 1997).

1.2.3 General Effects

The impact of growth hormones on both live animal performance and postmortem muscle have been very well documented. Schneider et al. (2007) reported that during the finishing periods, heifers implanted a single time had increased hot carcass weights (HCW) without negatively impacting marbling, grading scores or Warner-Bratlzer shear force (WBSF) values when

compared to non-implantedheifers. Additionally, Apple et al. (1991) reported that Holstein steers implanted with TBA, zeranol, or combined estradiol benzoate (EB) and progesterone, resulted in greater average daily gains (ADG) through the first 56 d versus those that did not receive an implant. Comparably, an increase in ADG and dry matter intake (DMI) was seen when feedlot steers were given a single combination implant containing an estrogen and an androgen (Duckett et al., 1997). Moreover, Reiling and Johnson (2003) reported that implanted cattle gained approximately 20% faster than non-implanted steers with significantly higher carcass weights, as well as less KPH fat and larger LM areas. Furthermore, heifers that were implanted a single time resulted in an increase in HCW, while not affecting the percentage of carcasses that graded USDA Choice or Prime (Schneider et al., 2007).

Overall, use of anabolic implant strategies have proven to be useful for improving the performance of growing and finishing cattle (Duckett and Andrae, 2001). However, apprehensions regarding the effect of implants on carcass quality grade and tenderness have been expressed within the literature (Smith et al., 2005b). Scanga et al. (1998) reported that implanted cattle had a higher incidence of dark cutters when compared to control animals. On the other hand, a review conducted by Duckett et al. (1997) reported that marbling score, yield grade (YG), QG, and incidence of dark cutters as well as WBSF values were not affected by implanting heifers a single time at the beginning of the finishing period. Moreover, implanting cattle at the production phases of weaning or branding only did not affect WBSF values (Platter et al., 2003); comparably, Gerken et al. (1995) found that there was also no effect on tenderness of strip loin steaks when given androgenic and combined implants. These studies also supported the findings from that of Belk and Savell (1992) which reported that implants containing TBA and estradiol also did not impact tenderness.

The combination implants involving estrogen and TBA have proven to increase fed cattle efficiencies such as feed to gain ratio, ADG, and DMI resulting in an increased final body weight when compared to cattle subjected to or non-implanted or single implant cattle (Apple et al., 1991; Perry et al., 1991; Duckett et al., 1997; Scheffler et al., 2003; Bruns et al., 2005). *Longissimus* muscle areas were also seen to increase with use of TBA (Gerken et al., 1995) and multiple implant combinations (Samber et al., 1996), along with improvement in HCW, LM area and DP when Revalor-S is administered (Baxa et al., 2010). On the other hand, Bruns et al. (2005) indicated that, regardless of implant treatment, there was no effect on dressing percent (DP), fat thickness, YG, LM area, or percentage of KPH fat for steers.

Samber et al. (1996) reported that implementation of two or more combination implants tend to produce steaks with WBSF values greater than those associated with non-implanted cattle. Additionally, greater overall shear force values were generated from cattle that were administered an estrogen implant (Garmyn and Miller, 2014). For all of the ten implant strategies employed by Platter et al. (2003), mean shear force values were 0.4 to 0.9 kg higher compared to non-implanted controls. Nonetheless, studies such as Huffman et al. (1991) reported no significance in WBSF values between steaks from control cattle compared to those that received single or combination implants. Regardless of the effects of meat quality and tenderness, use of anabolic implants, whether given in a single doses or combination strategies, are the most widely used form of growth promotion within finishing beef cattle (Arp, 2012).

1.3 Beta-adrenergic agonists

1.3.1 History

Beta-adrenergic agonists (βAA) belong to a class of compounds known as phenethanolamines, which are similar to the naturally occurring catecholamines (Scramlin et al., 2010). Catecholamines are most abundant in mammals as epinephrine, norepinephrine, and dopamine, and act through specific receptors on target tissues similar to hormones (Hossner, 2005). When considering the fundamental types of adrenergic receptors, they can be broken into two separate groups known as α and β receptors, with β-adrenergic receptors (βAR) being the most predominant receptor (Hossner, 2005). The βAA can bind to their corresponding βAR located on the cellular membrane to increase protein synthesis, decrease protein degradation, or both (Scramlin et al., 2010). While also being routinely used in livestock production, βAA are also utilized in human clinical medicine for conditions such as maintenance care of asthma, chronic obstructive pulmonary disease, and to stimulate cardiac contraction strength and rate (Hossner, 2005; Loneragan et al., 2014). β-agonists are utilized as feed supplements in food-animal production systems, where they have been proven to increase feed efficiency, live weight gain, carcass yields and leanness in cattle, swine and turkeys (Avendaño-Reyes et al., 2006; Ordóñez et al., 2009; Arp, 2012).

In the early 1980s Clenbuterol was discovered to be a metabolic modulator in cattle, chickens, pigs and sheep that increases muscle mass and decreases fat deposition (Ricks et al., 1984; Mersmann, 1998). In the following years, βAAs such as cimaterol, $L_{644,969}$, and salbutamol were administered to farm-animals (Mersmann, 1998). These were removed from the market later due to potential public health issues (Avendaño-Reyes et al., 2006). Soon after, Ractopamine

Hydrochloride (RAC; Paylean®; Elanco Animal Health, Greenfield, IN) was approved for use in swine in 1999 and in 2003 it was approved for use in cattle (Optaflexx®; Elanco Animal Health, Greenfield, IN). Three years later, Zilpaterol Hydrochloride (ZH; Zilamx®; Merk Corp., Summit, NJ) was approved for the administration to cattle in the United States). Use of ZH was previously approved in South Africa and Mexico. The percentage of fed cattle currently being administered βAA through major feeders is approximately 75 – 76 percent (B. Thoney, personal communication, February 27, 2020).

The βAAs bind to β receptors that can be subdivided into three subtypes: β1, β2, and β3. The βAR's are members of a family of G protein-coupled receptors that function in the regulation of energy distribution (Chikuni et al., 2008). β subtypes 1 and 2 have been very well researched within livestock species, with the evidence of a third subtype (β3) surfacing in the mid-1970s (Mersmann, 1998). The βAAs can bind to any of the three βARs. All three types of βARs are present in mammalian cells, but the concentration of each type varies depending on the specific tissue and specie (Mersmann, 1998; Johnson, 2004). While βAR's 1 and 2 are highly expressed throughout tissues in the body, βAR3 is found predominantly in adipocytes (Strosberg, 1997) and has been proposed to play an imperative role in lipid mobilization (Chikuni et al., 2008). Moreover, βAR3 has the greatest ability to induce lipolysis among all the three subtypes (Liggett and Raymond, 1993; Casteilla et al., 1994; McNeel and Mersmann, 1999). In addition to their role in lipid mobilization, βAR3 is present in skeletal muscle (Astrup et al., 1985), brown adipose tissue (Foster and Frydman, 1978), and white adipose tissue (Havel et al., 1964; Scheffler et al., 2003).

1.3.2 Mechanism of Action

Ractopamine is considered a category 1 βAA that increases growth and carcass characteristics (Pringle et al., 1993; Crome et al., 1996) through improvement of protein synthesis (Moody et al., 2000), while also having the ability to interact with both β1- and β2-adrenergic receptors (Hossner, 2005). Zilpaterol is considered a category 2 βAA that functions by increasing protein synthesis and decreasing protein degradation by interacting with β2 receptors (Mersmann, 1998; Moody et al., 2000).

The βAAs regulate cell metabolism through G_i protein stimulated cyclic-adenosine monophosphate (cAMP) mediated events (Johnson, 2004). All beta adrenergic receptors contain seven hydrophobic transmembrane domains and are members of the G coupled protein receptor family, which were modified to accommodate a variety of ligands (Mills and Mersmann, 1995). Each beta adrenergic receptor (βAR) contains extracellular segments which are associated with the N-terminus and intracellular loops associated with the C-terminus (Mills and Mersmann, 1995). Initiation of the βAA response occurs in C-terminal in the intracellular loop 3, which causes a conformational change to the receptors (Johnson, 2004). Binding of the βAAs to the α-subunit of the G_s protein initiates production of adenylate cyclase (AC), which increases production of cAMP, the primary intracellular signaling molecule (Mersmann, 1998). Cyclic AMP binds to the regulatory subunit of protein kinase A (PKA) that releases the catalytic subunit responsible for phosphorylation of necessary intracellular proteins involved in lipid and protein synthesis (Johnson, 2004). PKA then phosphorylates cAMP response element-binding protein (CREB) upregulating gene transcription (Mersmann, 1998). Phosphorylation that occurs as a consequence of stimulation of PKA provides the foundation for βAR-mediated transcription of genes located within the cell (Mersmann, 1998). Furthermore, βAR may permanently or temporarily retreat from

the cell surface if chronic βAA stimulation occurs (Ostrowski et al., 1992; Schwinn, 1992; Strosberg, 1992; Kobilka and Hoffman, 1995; Mersmann, 1998), leading to potential desensitization or inactivation of the βAR's on specific target tissue. Inactivation may also be a result of a limited number of βAR on the target tissues, therefore reducing responsiveness of the administered agonist (Mersmann, 1998).

Specific βAAs may not be as effective on the target tissue βARs across all species. This may be due to the agonists' affinity for the associated receptors, the pairing of the agonist-receptor complex to the signal transduction system, and several factors that affect the delivery of a specific compound to the receptor locations. There is a greater abundance of β1 adrenergic receptor (AR) in porcine tissue (McNeel and Mersmann, 1999) and β2AR in bovine tissue (Johnson et al., 2014) which may allow for the more profound effects of RAC in swine and ZH in cattle. There has been evidence supporting the idea that the βAR2 is more rapidly desensitized than the β1AR (Lafontan, 1994; Langin et al., 1995; Marullo et al., 1995). Interestingly, βAR3 is distinctly different from that of the β1AR and β3AR with its structure being in the fourth intracellular loop rather than the third. This might limit the opportunities for inactivation produced by phosphorylation (Arch and Ainsworth, 1983; Strosberg, 1992; Emorine et al., 1994; Langin et al., 1995); therefore, allowing fewer chances for desensitization compared to the other two subtypes (Mersmann, 1998).

1.3.3 Live Animal Performance

Some of the most obvious effects of orally administered βAA in species such as cattle, sheep, and pigs, is an increase in muscle mass and a decrease in carcass fat (Mersmann, 1998). In a meta-analysis conducted by Lean et al. (2014), when cattle were fed ZH, there was an 8 kg increase in body weight (BW) and a 0.15 kg increase in ADG over control cattle. Several studies

have reported improvement in final BW, ADG and gain to feed ratio (G:F) with ZH supplementation (Elam et al., 2009; Plascencia et al., 1999, Avendaño-Reyes et al., 2006; Baxa et al., 2010; Beckett et al., 2009; Montgomery et al., 2009; Scramlin et al., 2010). In comparison, Avendaño-Reyes et al. (2006) reported a 24% increase in ADG and a significant decrease in dry matter intake (DMI) when cattle were supplemented with RAC. Lean et al. (2014) indicated an increase in BW and ADG by 8 kg and 0.19 kg/d, respectively with RAC supplementation. Moreover, there was an improvement in gain:feed ratio with RAC (Lean et al., 2014). Other studies have also reported similar improvements in BW with RAC supplementation (Vogel et al., 2009; Abney et al., 2007; Gruber et al., 2007).

Results of research studies assessing effectiveness of ZH and RAC on live animal performance and growth vary. Scramlin et al. (2010) indicated a 4.35 kg increase in final BW with the administration of RAC compared to ZH; however, when compared to control cattle, RAC and ZH increased final BW by 7.53 and 3.13 kg, respectively. Moreover, Strydom et al. (2009) indicated an increase in final BW by 7.2 kg with RAC when compared to ZH. Furthermore, a significant increase in ADG and feed conversion was denoted with the supplementation of RAC over ZH.

1.3.4 Effects on Carcass Characteristics

Studies investigating effects of βAA on carcass characteristics have been extensive; however, results are conflicting. In a conventional two implant study, Woerner et al. (2011) indicated that supplementation of RAC averaged over steers and heifers had no effect on hot carcass weight (HCW), kidney pelvic and heart fat (KPH), marbling scores (MS) or maturity; however, an increase in *longissimus* muscle area (LMA) was observed. Similarly, HCW, LMA, adjusted fat thickness (aFT), KPH, MS, yield grade (YG) and quality grade (QG) (Quinn et al.,

2007; Winterholler et al., 2008; Allen et al., 2009) and cutout percentage (Allen et al., 2009) were not affected by the administration RAC. On the other hand, Vogel et al. (2009) reported that RAC supplementation increased HCW and LMA, whereas YG remained the same. Abney et al. (2007) indicated that RAC resulted in heavier carcass weights. Similar results were reported by Boler et al. (2012) with improvement in HCW, LMA and DP due to RAC supplementation. Bryant et al. (2010) reported a dose dependent improvement in DP and LMA when RAC was supplemented at 200 mg/hd/d, whereas those fed 100 mg/hd/d produced similar results as the controls. Moreover, RAC supplemented steers (200 mg/hd/d) and heifers (250 mg/hd/d) produced heavier carcass weights compared to the controls (Bryant et al., 2010), along with steers producing increased HCW over heifers (Bryant et al., 2010; Woerner et al., 2011). However, Quinn et al. (2007) reported no effect of RAC (200 mg/hd/d) on any carcass traits or MS when administered to finishing heifers.

Zilpaterol Hydrochloride and RAC supplementation have resulted in the improvement of HCW over that of control steers (Avendaño-Reyes et al., 2006). Van Donkersgoed et al. (2011), Scramlin et al. (2010), Garmyn et al. (2014) and Strydom et al. (2009) indicated carcass weights to be heavier from steers fed ZH than RAC. Studies observing effects of ZH on HCW reported that treated cattle produced heavier carcasses than the controls (Plascencia et al., 1999; Kellermeier et al., 2009; Montgomery et al., 2009; Baxa et al., 2010); whereas, Hilton et al. (2009) did not indicate any differences in carcass weight. Furthermore, LMA from carcasses that were supplemented with ZH were significantly larger than the controls (Plascencia et al., 1999; Avendaño-Reyes et al., 2006; Kellermeier et al., 2009; Strydom et al., 2009; Baxa et al., 2010).When both RAC and ZH were administered, steers that were fed ZH produced LMA that were significantly larger than those from RAC supplemented and non-supplemented steers (Scramlin et al., 2010; Garmyn et al., 2014). However, when ZH was fed for the final 20 and 40 d

of the finishing period, LMA increased as the feeding duration was extended and were larger than those from negative control steers and heifers (Montgomery et al., 2009). A multitude of studies have reported an increase in DP due to ZH supplement in steers (Plascencia et al., 1999; Avendaño-Reyes et al., 2006; Vasconcelos et al., 2008; Montgomery et al., 2009; Strydom et al., 2009; Baxa et al., 2010; Hilton et al., 2010; Scramlin et al., 2010) and heifers (Montgomery et al., 2009).

Results concerning the effect of ZH supplementation on aFT are contradicting, with several studies indicating no difference (Plascencia et al., 1999; Montgomery et al., 2009; Garmyn et al., 2010) and others stating a decrease in aFT with the inclusion of ZH (Vasconcelos et al., 2008; Strydom et al., 2009; Baxa et al., 2010; Scramlin et al., 2010; Garmyn et al., 2014) over extended feeding durations (Kellermeier et al., 2009). Overall, ZH has been reported to have no effect on KPH compared to carcasses from non-supplemented steers (Plascencia et al., 1999; Kellermeier et al., 2009; Montgomery et al., 2009; Strydom et al., 2009; Garmyn et al., 2010; Garmyn et al., 2014) with a select few who indicated a reduction (Baxa et al., 2010; Scramlin et al., 2010). Hot carcass weights, LMA, aFT and KPH all play a crucial role in determining overall YG; hence, as carcass weight and LMA increase and aFT and KPH decrease, there is an inevitable reduction in YG. Numerous studies have reported decreased YG due to the factors previously mentioned (Vasconcelos et al., 2008; Kellermeier et al., 2009; Montgomery et al., 2009; Strydom et al., 2009; Hilton et al., 2010; Scramlin et al., 2010; Garmyn et al., 2014).

Vasconcelos et al. (2008) supplemented ZH to finishing steers for the final 0, 20, 30 or 40 d of the finishing period, which resulted in a decrease of carcasses grading USDA Premium Choice and Choice, similar to the results published by Kellermeier et al. (2009). A majority of publications reported a decrease in MS due to the supplementation of ZH (Vasconcelos et al., 2008; Kellermeier et al., 2009; Montgomery et al., 2009; Strydom et al., 2009; Hilton et al., 2010; Scramlin et al.,

2010; Garmyn et al., 2014). However, MS from Vasconcelos et al. (2008) and Montgomery et al. (2009) were greater (Small) than those from Kellermeier et al. (2009) and Baxa et al. (2010) (Slight), with no differences reported by Plascencia et al. (1999).

1.3.5 Effects on Meat Quality

Supplementation of βAA can have a potential negative impact on meat quality attributes such as tenderness, measured using either shear force values or through consumer and trained sensory panels. Postmortem aging is a common practice in beef industry to improve tenderness and research has shown it can mitigate some on the negative impacts on tenderness (Scramlin et al., 2010; Boler et al., 2012). In addition, the impact on meat quality is largely dependent on dosage, feeding duration, sex, as well as breed classification.

1.3.5.1 Warner-Bratzler and Slice Shear Force

Warner-Bratzler Shear Force (WBSF) and Slice Shear Force (SSF) were greater in LL steaks from steers that were fed 200 mg/hd/d of RAC for the final 28 d of the finishing period compared to steaks from non-supplemented cattle (Gruber et al., 2008). However, these researchers, along with several other researchers, have reported a linear reduction in shear force values with aging (Gruber et al., 2008; Kellermeier et al., 2009; Rathmann et al., 2009; Scramlin et al., 2010). When cattle were supplemented with RAC, increased WBSF values were observed when steaks were aged for 3 and 7 d postmortem; however, there were no differences observed once 14 d of postmortem aging was achieved. Similar results were reported by Boler et al. (2012) with greater shear force values for RAC supplemented steaks compared to non-supplemented steaks. As with the other studies, this difference in tenderness was resolved with aging as there was no difference between RAC and control on days 7, 14, 21, and 28 of aging.

Zilpaterol Hydrochloride supplementation of steers and heifers for the final 20 and 40 d of the finishing period resulted in a 22 and 24% increase in WBSF values of steaks compared to steaks derived from the controls (Leheska et al., 2009). Similar increases in WBSF values were reported by Rathmann et al. (2009) whom noted that, as the feeding duration of ZH increased, steaks become less tender; however, regardless of the implemented aging periods treated steaks required the most force to shear compared to the controls (Kellermeier et al., 2009; Rathmann et al., 2009; Shook et al., 2009; Strydom et al., 2009; Hilton et al., 2010; Scramlin et al., 2010). Rathmann et al. (2009) indicated a linear aging response for the 0, 20 and 30 d ZH treatments. However, a reduction in WBSF values was observed as aging increased from 7 to 21 d (Kellermeier et al., 2009; Rathmann et al., 2009). Opposing results were reported by Van Donkersgoed et al. (2011) where no differences in tenderness among steaks derived from carcasses of cattle that were subjected to treatment with RAC or ZH occurred, and increased postmortem aging resulted in decreased WBSF values for steaks from heifers fed ZH or RAC.

Miller et al. (2001) determined that 100% of consumers found strip loin steaks to be acceptable in tenderness when WBSF values of < 3.0 kg were obtained for steaks. Compared to steaks produced from ZH supplemented cattle, the controls produced a higher proportion of steaks that were below the tenderness threshold of 3.0 kg after 7 d aging whereas the proportion of steaks that were aged for 7 d produced WBSF values that were below 4.3 kg (Rathmann et al., 2009). Additionally, as postmortem (PM) aging time increased from 14 to 21 d, the frequency of steaks considered tender (<4.4 kg) and very tender (<3.9 kg) increased for RAC and ZH (Garmyn et al., 2014). Furthermore, at 14 and 21 d of postmortem aging, ZH steaks required the most force to shear through compared to steaks from RAC, followed by control steaks (Garmyn et al., 2014). Moreover, in relation to SSF values, much like that of WBSF, ZH supplementation resulted in a

lower percentage of steaks considered very tender (15.3 kg) and tender (20.0 kg; ASTM, 2011). Garmyn et al. (2014) reported that RAC had the strongest response to PM aging compared to ZH or control; whereas the lowest WBSF values were seen by RAC steaks at 21 d of aging when compared to all other treatments.

1.3.5.2 Trained Sensory Evaluation

Trained panelists reported that steaks associated with the administration of ZH were significantly less tender than control steaks, with the highest dose of RAC (400 mg/hd/d) ranking similar to ZH steaks (Arp, 2012). In addition, panelists indicated that control steaks and steaks associated with the lowest dose of RAC (200 mg/hd/d) resulted in no differences in tenderness. Moreover, βAA had little to no detrimental effect on juiciness, beef flavor, and off flavors (Arp, 2012). Additionally, there was a tendency for ZH to decrease initial juiciness while a significant decrease was observed for sustained juiciness; nonetheless, no differences were reported for any flavor attributes (Garmyn et al., 2010). Steaks produced from ZH supplemented cattle resulted in decreased panel scores for tenderness and juiciness; whereas, overall juiciness scores tended to decreased as the duration of ZH supplementation increased from the last 20 to 40 d of the finishing period (Leheska et al., 2009). In addition, when panelists evaluated steaks produced from heifers rather than steers, scores for overall juiciness, flavor intensity and beef flavor were significantly reduced (Leheska et al., 2009). Gruber et al. (2008) denoted that panelists reported decreased rating for tenderness and juiciness.

1.3.5.3 Color

The initial indicator for consumer purchase acceptability is fresh lean color, with the perception that bright cherry red denotes freshness and quality. The βAA supplementation has

been reported to increase redness (a* value) when compared to non-supplemented cattle (Avendaño-Reyes et al., 2006). Martin et al. (2012) denoted that the addition of ZH to fed cattle diets ultimately resulted in steaks that were less red (lower a* value) and less vivid (decreased saturation) than control or those associated with RAC supplementation. However, when ZH was administered, no effect on b* value (yellowness) was reported when compared to the controls (Avendaño-Reyes et al., 2006). However, Garmyn et al. (2014) indicated that redness and yellowness did not differ between treatment groups (RAC and ZH); whereas, control cattle produced lean that was more red and more yellow. Furthermore, ZH supplementation resulted in lean that was lighter than that of RAC and controls (Garmyn et al., 2014). Avendaño-Reyes et al. (2006) reported a treatment effect for hue angle indicating that RAC and ZH resulted in increased redness of the LM compared to the controls. Some studies have suggested that RAC (Quinn et al., 2008; Woerner et al., 2011) and ZH (Rogers et al., 2010) does not affect a* (redness), b* (yellowness) or L* (lightness).

1.4 Immunohistochemistry of muscle fiber typing

Characteristics of fiber types vary with differences in oxidative and glycolytic metabolism, fiber size, color, glycogen and lipid contents (Schiaffino and Reggiani, 1996; Klont et al., 1998; Karlsson et al., 1999). Therefore, fiber type composition has the ability to influence post-mortem changes during meat conversion as well as their effects on meat quality (Essèn-Gustavsson, 1993; Karlsson, 1995; Maltin et al., 1997; Klont et al., 1998; Karlsson et al., 1999).

1.4.1 Muscle Fiber Classification

Skeletal muscle most commonly consists of four myosin chain isoforms identified as I, IIA, IIX and IIB (Gonzalez, 2008). Type I and IIA fibers are also known as red muscle fibers for

which metabolism is primarily oxidative, whereas Type IIX and IIB, otherwise known as white muscle fibers, primarily metabolize glycogen. Red muscle fibers are known to have greater amount of myoglobin and mitochondria, as well as have a superior lipid content, and have smaller cross-section areas than WMF (CSA; Listrat et al., 2016). However, white muscle fibers (IIX and IIB) are known to have a fast contraction speed leading to their susceptibility to fatigue, unlike red muscle fibers (I and IIA) whose slow contraction speeds results in less fatigue resistance. Furthermore, fiber types are classified into three subtypes: slow oxidative; SO (Type I), fast oxidative glycolytic; FOG (Type IIA) and fast glycolytic; FG (Type IIX); ultimately representing their characteristics. Myofibers are generally understood to represent approximately 75 – 90 % of the muscle volume (Lefaucheur, 2010), whereas at birth, the approximate fibers that make up the muscle bundle are 40 – 50% type IIB, 35 – 45% type IIA and 10 – 20% type I in cattle (Gonzalez, 2008).

1.4.2 Beta-adrenergic agonist influence on muscle fiber type

Cattle within confinement systems have been reported to have reduced potential for oxidative metabolism than those in extensive production systems (Vestergaard et al., 2000). Furthermore, nutritional supplementation has also been known to affect muscle fiber type shift (Gonzalez, 2008). Ractopamine supplementation in cattle has been observed to shift muscle fiber type from I to IIA (Seideman and Crouse, 1986; Gonzalez et al., 2009; Kellermeier et al., 2009; Garmyn et al., 2014; Kim, 2018). A previous study researching the effect of RAC on muscle fiber type revealed that the muscle was comprised of both type I and IIA fiber types, with no immunoreactivity of type IIB (Gonzalez et al., 2007). Whereas, when RAC was added in swine finishing rations there was an increase in the percentage of type IIB over type IIA and IIX fibers (Aalhus et al., 1992; Gonzalez et al., 2009). Depreux et al. (2002) and Gunawan et al. (2007)

agreed and indicated that RAC supplemented (100 mg/hd/d) hogs, muscle fiber type shifted from slow to fast-twitch glycolytic fibers; however, as dosage increased (200 mg/hd/d), a greater response was observed with approximately 30% of fibers shifting from type I to type II (Depreux et al., 2002; Gunawan et al., 2007).

Gonzalez et al. (2007) reported an increase in type I muscle fiber cross sectional area (CSA) with RAC administration in cull beef cows; however, no effect was observed on type II CSA. A later study by Gonzalez et al. (2009) reported that both type I and II muscle fiber CSA were not affected by RAC. Overall, it has been reported that there is an inability of RAC to shift muscle fibers to type IIB in cattle, indicating that fiber type shifts in response to RAC may only occur in muscles that initially expresses limited amounts of IIB (Gonzalez et al., 2007).

Baxa et al. (2010) indicated that ZH did not alter the concentration of type I or type IIA fibers; but previous research indicated that the change in muscle fiber diameter is a result of ZH supplementation (Baxa, 2008; Kellermeier et al., 2009). Use of ZH also increased the concentration of muscle fibers type IIX (Kellermeier et al., 2009; Baxa et al., 2010). In a study where ZH, clenbuterol (CL), and RAC were fed at 6, 2, and 30 ppm, it was observed that FG CSA was increased by 25% for CL and 17% for RAC and ZH compared to the control, whereas SO CSA where not effected by either treatment (Strydom et al., 2009). In addition, Korn et al. (2013) reported that ZH supplementation decreased the expression of type I and type II muscle fibers compared to controls. In contrast to these results, Rathmann et al. (2009) indicated that the type I and type IIX muscle fibers were not altered, though a decrease in the expression of type IIA muscle fibers were reported with the supplementation of ZH.

1.4.3 Effect of muscle fiber type on meat quality

Composition of muscle fibers can influence a number of meat quality attributes such as color, water holding capacity, tenderness, juiciness and flavor (Lefaucheur, 2010). However, identifying the particular affiliations between meat quality and myofiber characteristics (Lefaucheur, 2010) or muscle fiber CSA is highly challenging (Seideman and Crouse, 1986; Renand et al., 2001).

Some studies have shown that a greater proportion of FOG fibers can decrease WHC, tenderness, juiciness, and flavor of cooked meat (Henckel et al., 1997; Maltin et al., 1997). Additionally, specific properties such as CSA could contribute to differences in cooking loss (Garmyn et al., 2014; Cannon et al., 1995). Waritthitham et al. (2010) confirmed a positive relationship between CSA and cooking loss for both slow and fast-twitch fibers.; whereas, Ozawa et al. (2000) indicated that fiber diameter of type IIA and IIB fibers had no relationship. Moreover, the increase in the rate and extent post mortem pH decline, lightness (L*), paleness, cooking loss, and protein degradation in hogs have been associated with an increase in fast-twitch glycolytic fibers (IIB; Larzul et al., 1997; Kauffman et al., 1998; Ozawa et al., 2000; Rosenvold et al., 2001; Ryu and Kim, 2005; Choi et al., 2006; Ryu and Kim, 2006; Choi et al., 2007). A general accepted theory for the effects of βAA on tenderness is the change in muscle fiber diameter (Arp, 2012) and muscle fiber type (Calkins et al., 1981), along with a hypothesis that ZH supplementation and increased WBSF values can be attributed to the increased fiber diameter (Kellermeier et al., 2009).

1.5 Proteome Analysis

According to Wilkins et al. (1996) the term proteome is defined as the protein complement of a genome; thus, proteomics refers to characterizing the complements of proteins that are

expressed in a cell or tissue type (Bendixen, 2005a). The aim of proteomics is to be able to identify all proteins, their biological activity, post-translational modifications and interactions in a cell and to identify changes in the proteome response to altered biological conditions (Zamaratskaia and Li, 2017). The additional objective of proteomics is to discover molecular markers that would allow for a more accurate understanding of particular mechanisms (Bendixen, 2005a). The proteome can be perceived as the molecular relationship between the genome and the functional quality characteristics of meat under certain environmental and processing systems (Hollung et al., 2007).

Meat quality is directly affected by pre- and post- harvest conditions as well as processing and storage systems; subsequently, proteomics is a promising approach in gaining knowledge on the underlying mechanisms that both directly and indirectly affect meat quality (Zamaratskaia and Li, 2017). Throughout the last decade, the applicable research approach to proteomics has considerably increased in regard to postmortem protein changes. Methodologies such as affinity, exchange and size exclusion chromatography are technologies utilized for protein purification, whereas enzyme linked immunosorbent assay and western blotting are used for the evaluation of selective proteins (Nair and Zhai, 2020).

1.6 Proteomics in meat quality

Fresh meat color, texture, and tenderness are key attributes that influence purchasing and re-purchasing decisions of consumers. The underlying mechanisms that effect these characteristics are still not fully understood; therefore, innovative techniques within the realm of proteomics have been extensively applied to meat science research in order to better understand and explain the molecular mechanisms that control meat quality attributes such as color (Sayd et al., 2006; Suman et al., 2007; Joseph et al., 2012) and tenderness (Jia et al., 2009; Anderson et al., 2014; Picard et

al., 2014). Bendixen (2005a) and Bendixen (2005b) suggested that the ability to understand the analytical components and characteristics of the proteome in relation to meat quality and processing systems will ultimately lead to a better understanding and a greater ability to optimize the conversion of muscle to meat.

1.6.1 Color

Recent advancements in proteome technologies and bioinformatics have been utilized to explain the fundamental mechanism of meat color (Canto et al., 2015). Sarcoplasmic protein such as myoglobin including other enzymes constitute approximately 30% of skeletal muscle and are highly influential to meat color (Nair and Zhai, 2020). Although numerous pre- and post-harvest procedures contribute to shelf stable meat color (McKenna et al., 2005; Seyfert et al., 2006; Seyfert et al., 2007), the interactions that occur between myoglobin and sarcoplasm proteins are critical to the stability of fresh meat color (Joseph et al., 2012).

Utilization of 4-hydroxy-2-nonenal (HNE) as an aldehyde model on myoglobin redox reactions and the effect of lipid oxidation was a large scope of the early color research involving proteomics (Nair and Zhai, 2020). The covalent modification of histidine in myoglobin of pork (Lee et al., 2003) and beef (Alderton et al., 2003) was considered to be responsible for discoloration of fresh meats. Beef muscles show muscle-specificity in meat color and could be categorized on the basis of retail color stability as color stable and color liable (Joseph et al., 2012) with the *longissimus lumborum* (LL) being relatively color-stable and is one of the most extensively studied in regards the biochemistry of meat color (Canto et al., 2015). Canto et al. (2015) reported similar results suggesting that the difference in color stability observed between color labile and color stable beef LL muscles could be due to the difference in the proteome profile.

1.6.2 Tenderness

Proteins that play a pivotal role in tenderness are involved in glycolysis and energy metabolism, heat shock, oxidative stress resistance, myofibril structure and proteolysis (Paredi et al., 2012). Several studies have correlated tenderness with a series of heat shock proteins (HSPs), which include DNAJA1 (HSP40; Bachi and Bonaldi, 2008), HSPB1, (HSP27; Morzel et al., 2008), HSP70, HSPA8, α-crystallin (CRYAB) and a number of other chaperone proteins (Guillemin et al., 2011). Increased tenderness scores have been suggested to be correlated with a higher expression of HSP27, along with succinate dehydrogenase being a predictor of initial and overall tenderness (Nair and Zhai, 2020). However, Paredi et al. (2012) suggested that four differentially expressed proteins (HSP27, chaperone protein containing T-complex protein 1 and inositol 1,4,5-triphosphate receptor type 1) to be associated with decreased tenderness (Paredi et al., 2012). Nonetheless, Kim et al. (2008) reported that decreased levels of HSP27 were found in beef muscle that was had increased tenderness. Similarly, peroxiredoxin-6 was found to be over-abundant in tender meat as it contributes to detoxification of reactive oxygen species (Paredi et al., 2012). Lametsch et al. (2003) researched the proteome changes in pork LD muscle in regardrelative to tenderness. Six differential proteins were observed to be correlated with shear force (three actin fragments and a myosin heavy chain fragment) and tenderness (myosin light chain II and triose phosphate isomerase) which agreed with results from Hwang et al. (2005). Several studies reported that a higher expression of protein fragments associated with actin, myosin heavy chain and myosin light chain 2 were correlated with increased tenderness (Lametsch et al., 2003; Bouley et al., 2004; Kim et al., 2008).

1.6.3 Water Holding Capacity

Water holding capacity (WHC) is one of the most important factors contributing to meat quality as it related to juiciness, texture and visual appearance. Pale lean with decreased WHC has been associated with the solubilization of myofibrillar proteins and the insolubilization of sarcoplasmic proteins (Marcos and Mullen, 2014), indicating that sarcoplasmic proteins are crucial when determining WHC. Zuo et al. (2018) indicated that over-expression of desmin, troponin-T, and L-lactate dehydrogenase were associated with high cooking loss in steaks. Similar proteins and enzymes identified in the previous study were also reported by Zhang et al. (2019) with the addition of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and peroxidoxin-1 that were found to be different between steaks with low and high drip loss. Research on chicken pectoralis muscle with differing WHCs indicated that the over expression of triosephosphate isomerase and heat shock proteins were indicative of low and high WHC, respectively? (Phongpa-Ngan et al., 2011), along with creatine kinase later being deemed as a possible biomarker for WHC in low and high drip loss in pork muscle (Van de Wiel and Zhang, 2007). Pale, soft and exudative meat is associated with poor WHC and had over-abundance of protein biomarkers such as actin alpha, myosin heavy chain, phosphoglycerate kinase, creatine kinase M type, β-enolase, carbonic anhydrase 2, proteasome subunit alpha, pyruvate kinase, and malate dehydrogenase (Desai et al., 2016).

1.7 Beta agonists and proteomics

The literature addressing effects of βAAs on proteome profile mostly have focused on RAC supplementation in hogs (Costa-Lima et al., 2015; Wu et al., 2017), whereas one study focused on supplementation of RAC in beef steers (Kim et al., 2017).

Both Costa-Lima et al. (2015) and Wu et al. (2017) reported Myosin light chain 1/3 (MLC1/3) as being over-abundant in RAC treated muscle. Similarly, L-lactate dehydrogenase A chain (LDHA) was more abundant in RAC supplemented muscles (Costa-Lima et al., 2015; Kim et al., 2017). Wu et al. (2017) reported six differentially abundant proteins between control and RAC supplemented pork *semimembranosus* muscle, with 4 being over-abundant in control (Hemoglobin subunit β; HEB, alpha-crystallin B chain; CRYAB, titan fragment) and 2 being over-abundant in RAC (MLC1/3 and tripartite motif-containing protein 72; TRIM72). These authors suggested that the over-abundance of HEB seen in the control group could possibly be attributed to the shift from oxidative to glycolytic metabolism, or Type I to Type II muscle fiber types that is commonly seen with RAC supplementation (Aalhus et al., 1992; Depreux et al., 2002; Gunawan et al., 2007).

Costa-Lima et al. (2015) reported seven differential proteins with RAC supplementation with two being over-abundant in control (glyceraldehyde-3-phosphate dehydrogenase; G3PDH, phosphoglucomutase-1; PGM1) and five being over-abundant in RAC (serum albumin; SA, carbonic anhydrase-3; CA3, fructose-biphosphate aldolase A; ALDOA, LDHA; MLC1/3) *longissimus thoracis* (LT) muscle. The protein G3PDH has been reported to be positively correlated to redness (a* value) and color stability (Canto et al., 2015); as well as to fast-twitch Type II muscle fibers (Okumura et al., 2005). Additionally, cytosolic G3PDH (cG3PDH) plays a critical role in glycolysis by oxidizing NADH and H^+ into NAD^+ (Holloszy and Booth, 1976; Haddock and Jones, 1977). Phosphoglucomutase-1 is a key regulatory enzyme involved in glycogen metabolism mediating the conversion of glucose-1-phosphate and glucose-6-phosphate to glucose-1,6-bisphosphate (Cori and Cori, 1936; Hwang et al., 2005); whereas, ALDOA is responsible for catalyzing the conversion (Tochio et al., 2010). It has also been reported that an

PGM1 is more abundant in tender meat than tough meat (Picard et al., 2014), suggesting that the expression of PGM1 is related to tenderness (Anderson et al., 2014). According to Bergen et al. (1989) and Mills et al. (2003), the over-abundance of SA that was seen in pork *longissimus* thoracis muscle could have been due to the increase in protein accretion and lipolysis that is seen in the muscles of RAC fed livestock. Moreover, CA3 has been suggested as a biomarker for pork quality (Hwang et al., 2005) and has been positively correlated with L* values (Damon et al., 2013). A higher expression of LDHA was observed in low quality than high quality swine *longissimus* dorsi (LD) muscle (Choe et al., 2008), and is also known for catalyzing the conversion of pyruvate to lactate during glycolysis (Fan et al., 2011).

Kim et al. (2017) identified 5 differential proteins between control and RAC fed steers. These proteins have been associated with muscle contraction development (F-actin capping protein subunit beta-2, PDZ and LIM domain 3), chaperone activity (heat shock protein beta 1, HSP27), oxygen transport (myoglobin), and glycolysis (LDHA). As described earlier, the expression of HSP27 has been associated with color (Sayd et al., 2006; Joseph et al., 2012) and tenderness (Kim et al., 2008; Carvalho et al., 2014) and has also been found to be over-abundant in muscle hypertrophy during compensatory growth (Lametsch et al., 2006).

Overall, several research studies has examined the impact of growth promoting technologies such as beta-agonists on live animal growth performance, meat quality, and proteomics. The objective of current research was to evaluate a novel β-3 modifier on carcass characteristics, muscle fiber type, muscle proteome, and meat quality of finished feedlot steers.

CHAPTER 2 - AN EVALUATION OF THE SUPPLEMENTATION OF LUBABEGRON ON CARCASS CHARACTERSITICS OF FINISHED FEEDLOT STEERS

2.1 Introduction

The current state of livestock production and the economy demands an increased use of technology to make beef and pork production more efficient and affordable (Arp, 2012). Among the several technological improvements, an alternative class of growth promotants known as β-adrenergic agonists (βAA) have thrived within the livestock industry over the past decade due to the drastic increase in performance and carcass yields that they generate (Garmyn and Miller, 2014). Beta- adrenergic agonists, otherwise known as repartitioning agents, are technologies that producers have continued to utilize in order to increase productivity, while amplifying efficiency and overall production yields. Approvals by FDA for use of βAA's such as Zilpaterol hydrochloride (Zilmax®, Merck Animal Health) and Ractopamine hydrochloride (Optaflexx®, Elanco Animal Health) have allowed cattle feeders to combine growth-promoting effects of steroidal implants with repartitioning agents (Arp, 2012). The increased growth and performance can lead to increased skeletal muscle accretion and improved overall carcass yield (Perry et al., 1991; Kellermeier et al., 2009; Parr et al., 2011).

Beef tenderness is arguably one of the most important traits affecting the present and future of beef palatability (Dikeman, 1987; Savell et al., 1987; Miller et al., 1995; Miller et al., 2001). The use of both Ractopamine hydrochloride (RAC) and Zilpaterol hydrochloride (ZH) have shown overwhelming effects on hot carcass weight (HCW), *longissimus* muscle area (LMA), and carcass cutability (Gruber et al., 2007; Rathmann et al., 2009; Shook et al., 2009; Vogel et al., 2009; Hilton et al., 2010; Scramlin et al., 2010). Simultaneously, research has reported a negative effect of these

compounds on carcass quality traits (Reiling and Johnson, 2003), product tenderness (Schneider et al., 2007), marbling scores, incidence of dark cutters (Roeber et al., 2000), and consumer taste panel scores (Barham et al., 2003).

In addition, due to issues associated with animal welfare , major packers such as Cargill and Tyson Foods announced in 2013 that they would no longer be purchasing cattle that were supplemented with Zilpaterol Hydrochloride (ZH). Much later, JBS USA and Smithfield Foods eliminated the administration of RAC within their pork production facilities (Welshans, 2019) in order to capitailize on export opportunities as many countries such as China, European Union, and Russia have developed a zero tolerance policy for beta-agonist fed livestock. Therefore, the U.S. Beef and Food Industries need an alternative feed supplement that will maintain the current live weight gain, feed efficiency and lean carcass yields, while mitigating the potential negative impact on tenderness and marbling scores (Avendaño-Reyes et al., 2006; Gruber et al., 2007; Gonzalez et al., 2010) associated with other βAAs.

Both RAC and ZH are considered βAA which implies binding and activating either the β1 or β2 receptor without obtaining the capability to block or antagonize the other. The new feed supplement known as Lubabegron or "Experior", developed by Elanco, is instead a β-adrenergic agonist/antagonist (i.e., β-modulator), meaning that it activates a β3 receptor while blocking the β1 and β2 receptors. In addition, the β3 receptor (R) is distinctly different from the β1 and β2R's, with its structure being in the fourth intracellular loop rather than the third, and primarily contained within adipocytes (Fiems, 1987; Strosberg, 1992; Nisoli et al., 1996; Mersmann, 1998; Chikuni et al., 2008). Because of its location in the fourth loop, there are fewer opportunities for inactivation of the β3R binding site by phosphorylation (Arch and Ainsworth, 1983; Strosberg, 1992; Emorine et al., 1994; Langin et al., 1995), thus, reducing desensitization compared to the other two subtypes

(Mersmann, 1998).The objective of this study was to evaluate the effect of β3- adrenergic agonist/antagonist, Experior (EX), on carcass characteristics and muscle fiber typing.

2.2 Materials and Methods

2.2.1 Live Animal Phase

A total of 2,160 British or European continental crossbred steers were utilized for the study in a 4 x 3 factorial comprised of 2 factors; dose (0, 1.4, 3.2, 5.0 g/ton) and duration (28, 56, 84 d) in a complete randomized block design. Within each duration group (28, 56 and 84 d), 15 pens (12 hd/pen) were assigned to each dosage level (0, 1.4, 3.2, 5.0 g/ton), resulting in 60 pens per duration group and 180 total pens across all durations. Three blocks were assigned to each study cycle, where each block consisted of 12 pens (12 hd/pen, 3 pens per treatment group) resulting in a total of 432 steers (36 pens) for each study cycle. Once all five study cycles were complete, a total of 2,160 crossbred steers were subset and analyzed for carcass characteristics.

Weights were collected and recorded 8 days prior to one week and one day before the start of each treatment phase, and final weights were collected at the end of the treatment phase. Additionally, all steers included in this study were have never allowed to receive an implant or had to be explanted on or before day 99 (14 d prior to the start of the 84 d feeding duration group). All steers were fed a total mixed ration (TMR) including Monensin (40 g/ton) and Tylosin (8 g/ton) by day ~105 and were switched from a basal to a finishing ration at the start of the durational period (day ~85, day ~57, or day ~29) that would contain either 0, 1.4, 3.2 or 5.0 g/ton of LY488756 otherwise known as Lubabegron (Experior®, Elanco Animal Health; EX) (100% dry matter basis).

2.2.2 Product Collection and Aging

Cattle were processed in a commercial beef processing facility in Pasco, Washington over five harvest periods beginning July 11th, 2018 through September 5th, 2018. Steers were harvested during the second or "B" plant shift on the associated Wednesday of each harvest day. Cattle were harvested using the standard U.S. beef industry practice and USDA/ FSIS inspection criteria.

A total of 36 treatment pens (n = 36; N = 180) and 3 blocks (144 steers/block) were harvested consecutively during each one of the five harvest days and each carcass from each pen was able to be identified and traced to an individual plant sequence number that was maintained throughout the carcass data collection process, along with their hot carcass weight (HCW). Grade data such as HCW, adjusted fat thickness (aFT), Kidney, pelvic and heart fat (KPH) and *Longissimus* muscle area (LMA) was collected. Hot carcass weight, LMA and marbling scores were measured by USDA plant personnel, while, aFT and KPH were taken by trained Colorado State University Personnel.

A subset of three carcasses that graded USDA Choice were randomly selected from each treatment pen (n = 108; N = 540) for inclusion into the meat quality study. Objective color measurements were obtained using a portable Spectrophotometer (Hunter. MiniScan XE, Hunter Labs, Reston, VA) where L*, a* and b* measurements were collected. From each treatment group, 1.27-cm steaks were obtained from the *longissimus* muscle of eight animals (n = 8, N = 96) and were immediately placed in whirl-pak bags, placed over ice packs and shipped to Kansas State University were muscle fiber typing was conducted.

Following collection of grade and color data, a 6.35-cm section was pulled and frozen from the most anterior portion of all striploins to represent the day 1 postmortem aging period. All striploins from the right side of each carcass were fabricated where they were collected, labeled,

packaged, placed in boxes, put on pallets and shipped over dry ice in a refrigerated truck to the Meat Laboratory at Colorado State University (CSU) for further processing. Upon on arrival at CSU, the loins were fabricated into 5, 6.35-cm sections and randomly assigned to postmortem aging periods (1, 7, 14, 21, or 28 d). On the designated day of aging, sections were frozen at a temperature of -20°C. As seen in Figure 2.1, a bandsaw was later used to fabricate each 6.35-cm aged section into two 2.54-cm steaks and one 1.27-cm section. Within each aging period, one 2.54-cm steak was assigned to a shear force; whereas only those aged for 14 d were both 2.54-cm steaks assigned to a shear force and a trained sensory evaluation day. Additionally, the 1.27-cm sections from the appropriate treatments from those aged for 1 d were assigned to proteomics.

2.2.3 Carcass Characteristics

Hot carcass weights, preliminary yield grades (PYG; later converted to aFT), KPH as well as lean and skeletal maturities were collected by trained Colorado State University personnel. Meanwhile, at the USDA grading station, carcass selection was determined by the random selection of three USDA Choice carcasses within each pen and were rolled onto the regrade rails. Immediately after carcasses selection, a USDA grade camera depicted LMA and PYG. Once placed onto the regrade rails, the objective color was recorded by trained CSU Personnel. Using the appropriate factors (aFT, HCW, LMA, KPH), USDA yield grades (YG) were later calculated for each carcass.

2.2.4 Dimensional Steak Measurements

Steaks that were used for tenderness evaluation also were used for steak dimensional measurements. An image of each steak from all treatment combinations was obtained using a digital camera with a fixed zoom lens that was attached to a tripod. This allowed for all images to

be directly collected from above each steak and in order for the distance to remain consistent. Each individual steak was placed on a clean, 2.54-cm gridded background with their associated tag in order to maintain the identity. All individual images were downloaded and analyzed using an image software system (ImageJ, Wayne Rasband) capable of quantifying pixel size per inch of an image. The total area proportion of lean was measured for all individual images. Following image capture, steaks were subjected to tenderness evaluation.

2.2.5 Statistical Analysis

Separate mixed models were fit using multiple response variables, including those from carcass traits (HCW, aFT, KPH, LMA, YG, marbling score (MS)). Fixed effects included dose (0, 1.4, 3.2, 5.0 g/ton; 100% dry matter) and feeding duration (28, 56, 84 d) plus the dose*feeding duration interaction. Pen was used as a random effect along with block being nested within cycle. For each response variable, treatments were compared using Tukey adjusted pairwise comparisons. Carcasses with missing or unrecorded data were removed from the statistical analysis. Analysis was conducted using R Studio (RStudio 1.1.463) and the lme4 (Bates et al., 2015) lmertest (Kuznetsova et al., 2017) and emmeans (Lenth, 2019) packages with significance set at an alpha level of 0.05.

2.3 Results and Discussion

2.3.1 Carcass Characteristics

Table 2.1 depicts carcass traits from all the carcass data (N = 2114). No two-way interactions ($P > 0.05$) between dose x feeding duration (FD) were detected for any characteristics

analyzed. The main effect of dose ($P < 0.05$) influenced HCW, LMA, MS and YG. Additionally, the main effect of FD ($P < 0.05$) affected HCW and marbling score (MS).

Our results were similar to Montgomery et al. (2009), where no interaction was observed between ZH dosage and feeding duration when administered to either steers or heifers. In the present study, the supplementation of EX increased ($P < 0.05$) carcass weight by approximately 10.2 kg compared to the controls. As feeding duration increased from 28 to 56 and 84 d, carcass weights increased by approximately 4.12 kg. A study where RAC dosage increased from 200 to 300 mg/hd/d there was a 4.7 and 5.1 kg improvement in HCW (Vogel et al., 2009), where when fed 200 mg/hd/d only an increase of 1.68 kg was observed (Boler et al. (2012). In the current study, HCW improved 8.7, 10.5, and 11.4 kg as dose increased from 1.4, 3.2 to 5.0 g/ton. On the contrary, Woerner et al. (2011) and Gruber et al. (2007) reported that HCW were unaffected by the administration of RAC. When RAC was supplemented at 300 and 400 mg/hd/d to cattle, carcass weights improved when compared to those fed 200 mg/hd/d and the control diet (Arp et al., 2014). Bryant et al. (2010) reported a 1.0 (363.1 kg) and 6.3 (368.4 kg) kg increase in HCW as RAC dosage increased from 100 to 200 mg/hd/d compared to controls. In addition, Allen et al. (2009) denoted a 6.5 kg improvement in carcass weight when cattle were supplemented with RAC. Whereas, Van Donkersgoed et al. (2011), Scramlin et al. (2010) and Strydom et al. (2009) indicated carcass weights to be 9.7, 7.62, and 7.4 kg heavier from steers fed ZH than RAC.

Vogel et al. (2009) reported a 2.78 cm^2 improvement in LMA from steers that were supplemented with 300 mg/hd/d as to those administered 200 mg/hd/d of RAC, with an associated 0.14 reduction in YG. Experior supplementation resulted in larger ($P < 0.05$) LMA when compared to the controls, coupled with a decrease ($P < 0.05$) in YG. Whereas Arp et al. (2014) reported that when RAC was supplemented to cattle at 200 and 300 mg/hd/d LMAs were similar to those of

non-supplemented cattle; however, a significant increase was seen when the dose was increased to 400 mg/hd/d. Yearling steers produced LMA that were 2.3 cm^2 larger when supplemented with 200 mg/hd/d of RAC compared to non-supplemented steers (84.0 cm^2), whereas those fed 100 mg/hd/d showed no increase in LMA (Bryant et al., 2010). Interestingly, EX resulted in the largest increase in LMA seen by the use of a βAA (Avendaño-Reyes et al., 2006; Quinn et al., 2007; Allen et al., 2009; Strydom et al., 2009; Vogel et al., 2009; Bryant et al., 2010; Garmyn et al., 2010; Arp, 2012; Boler et al., 2012) to date, excluding a small percentage of publications that reported LMA similar to those in the present study (Vasconcelos et al., 2008; Elam et al., 2009; Kellermeier et al., 2009; Woerner et al., 2011)

In the present study, when cattle were supplemented the highest dose of EX, MS decreased ($P < 0.05$) compared to the controls. However, when the low and medium doses were supplemented, cattle produced MS that were similar to those fed the control and high dose. Boler et al. (2012) reported a decrease of 17.9 and 27.7 degrees as the dosage of RAC increase from 200 to 300 mg/hd/d. Vogel et al. (2009) reported a 9.3 degree decrease with the inclusion of RAC as dosage increased from 200 to 300 mg/hd/d. The supplementation of RAC (300 mg/hd/d) produced MS that decreased 17 degrees compared to muscle from non-supplemented cattle (Arp et al., 2014). Kellermeier et al. (2009) reported a 40 degree reduction in MS when comparing ZH supplemented and non-supplemented cattle. In comparison, Baxa et al. (2010) and Garmyn et al. (2010) indicated a reduction in MS by 29.9 and 14.4 degrees in cattle supplemented with ZH over that of the controls.

A multitude of studies have reported marbling scores of USDA Low Choice (SM00-99) (Allen et al., 2009; Vogel et al., 2009; Garmyn et al., 2010; Scramlin et al., 2010; Woerner et al., 2011; Boler et al., 2012) as a result of beta-agonist supplementation. However, the current study

produced all USDA Premium Choice (MT00-99); whereas, Quinn et al. (2007) and Kellermeier et al. (2009) reported that supplementing RAC and ZH ultimately resulted in QG's of USDA Select rather than Choice. Contradicting results were reported by Arp et al. (2014), indicating the supplementation of RAC and ZH resulted in cattle grading USDA Low Choice (SM00-99). However, Strydom et al. (2009) indicated that, compared to the controls, QG for RAC remained as USDA Select and those supplemented ZH resulted in USDA Standard.

In the current study, feeding duration did not affect ($P > 0.05$) aFT or KPH, agreeing with the results reported by Plascencia et al. (1999) ; Arp et al., 2014). Similarly, Quinn et al. (2007), Winterholler et al. (2008), and Allen et al. (2009) reported that RAC supplementation did not result in a significant difference in aFT or KPH. Additionally, the supplementation of EX in the present study did not affect the percentage of. Kidney pelvic and heart fat. On contrary, Baxa et al. (2010) reported that ZH supplementation resulted in a 0.15 cm and 0.19 % decrease in aFT and KPH. However, Strydom et al. (2009) indicated that cattle supplemented with a β1AA produced an aFT that was very much similar to those not supplemented with a βAA. Furthermore, KPH increased by 1.21 kg (2.40 kg) with the supplementation of RAC over that of ZH (2.30 kg), where no statistical significance was observed between RAC (2.40 kg) and the controls (2.41 kg) (Scramlin et al., 2010). On contrary, Strydom et al. (2009) reported that the percentage of KPH was similar between RAC, ZH as well as the control.

2.3.2 Dimensional Steak Measurements

Longissimus muscle area (LMA) was measured using Image J and a USDA Grade Camera at a commercial beef processing facility. Results are presented in Figure 3.3. There was no two-way interaction between dose (g/ton) x feeding duration (FD) ($P = 0.497$); therefore, the main

effect of dose ($P < 0.05$) were used to construct the linear model for ANOVA testing. Interestingly, the LMA measurements obtained from the two methods differed by an average of 28.76 cm^2.

Image J resulted in LMA that were similar ($P > 0.05$) between all steaks that were produced from supplemented cattle, but statistically larger (122.68, 124. 52, 123.04 cm^2) than those generated from carcasses of control cattle (117.91 cm^2). *Longissimus* muscle area depicted by the USDA grade camera resulted in more variability with steaks from cattle fed 3.2g/ton being 4.21 cm^2 larger than the controls (90.97 cm^2). Additionally, cattle supplemented 1.4 (93.27 cm^2) and 5.0 (93.45 cm^2) g/ton produced LMA that were similar to all other treatment dosages.

2.2 Conclusions

Results from the current study indicate that Experior could be an effective tool to increase and maintain growth and sub primal yield. Experior did not affect perinephric or intermuscular fat deposition. However, the supplementation of Lubabegron increased carcass weights, *longissimus* muscle area, while simultaneously decreasing yield grade and marbling scores. Nonetheless, Experior produced yield grade 3 carcasses grading USDA Premium Choice.

Table 2.1. Effects of feeding 4 doses of Experior (EX; 0, 1.4, 3.2, 5.0 g/ton DM basis) for 3 feeding durations (28, 56, 84 d) on carcass characteristics of feedlot steers from all USDA quality grades (n = 2114).

Trait	Experior, g/ton dry matter (DM)				P-value	SEM[1]	Feeding duration (FD)			P-value	SEM[1]
	0	1.4	3.2	5.0			28	56	84		
HCW, kg[2]	389.6[b]	398.25[a]	400.1[a]	401.0[a]	<0.01	15.40	394.6[b]	398.3[a]	399.2[a]	<0.01	15.40
LM area, cm2[3]	88.9[b]	91.8[a]	93.0[a]	93.3[a]	<0.01	1.08	91.1	92.1	92.0	0.12	1.06
Adjusted fat thickness, inin	0.58	0.58	0.56	0.57	0.33	0.38	0.58	0.56	0.57	0.25	0.04
KPH,%[4]	1.85	1.88	1.85	1.84	0.78	0.23	1.84	1.84	1.89	0.15	0.23
Marbling score[5]	537.0[a]	524.0[ab]	520.0[ab]	515.0[b]	0.01	9.66	533.0[a]	523.0[ab]	516.0[b]	0.02	9.18
USDA YG	3.38[a]	3.29[ab]	3.26[b]	3.23[b]	<0.01	0.05	3.30	3.28	3.29	0.821	0.05

[ab] Within a row, values with a different superscripts are different ($P < 0.05$)

[1] Standard error of the means

[2] HCW - hot carcass weight

[3] LM area- longissimus muscle area

[4] KPH -kidney pelvic and heart fat percentage

[5] Marbling scores 300 = slight; 400 = small; 500 = modest

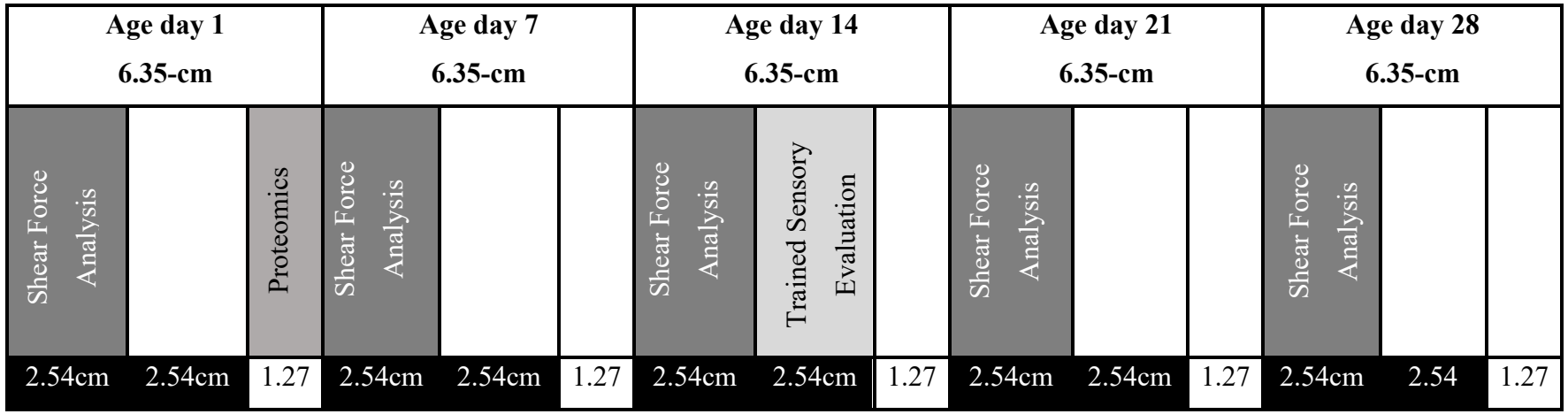

Figure 2.1. Schematic of striploin breakdown for aging and quality evaluation. The sections were randomly allocated to each of the aging period.

CHAPTER 3 - THE IMPACT OF LUBBEGRON SUPPLEMENTATION IN FINISHED FEEDLOT STEERS ON MEAT QUALITY AND TENDERNESS

3.1 Introduction

An increased use of technology to make beef and pork production more efficient and affordable is necessary in the current state of livestock production (Arp, 2012). Beef tenderness is arguably one of the most important traits affecting beef palatability (Dikeman, 1987; Savell et al., 1987; Miller et al., 1995; Miller et al., 2001). As tenderness highly influences consumer satisfaction and repurchasing decisions, it is pertinent that any technology to improve beef production efficiency do so without adversely affecting tenderness.

Among the several technological improvements, an alternative class of growth promotants known as β- adrenergic agonists (βAA) have thrived within the livestock industry over the past two decades due to the drastic increase in performance and carcass yields (Garmyn and Miller, 2014). Beta- adrenergic agonists, otherwise known as repartitioning agents, increase productivity gains, while amplifying yield and overall production efficiency. The majority of the research during the last decade has involved the usage of two βAAs: Zilpaterol hydrochloride (Zilmax®, Merck Animal Health; ZH) and Ractopamine hydrochloride (Optaflexx®, Elanco Animal Health; RAC). While these βAAs have proven their effectiveness on animal performance and carcass characteristics, there has been conflicting research on their effects on beef tenderness (Quinn et al., 2008; Allen et al., 2009; Rathmann et al., 2009; Scramlin et al., 2010; Boler et al., 2012).

Composition of muscle fibers has the ability to significantly influence a number of meat quality attributes such as color, water holding capacity, tenderness, juiciness and flavor (Lefaucheur, 2010). Cattle supplemented with Ractopamine Hydrochloride (RAC) demonstrated

a shift from type I to IIA, or those that rely on glycolytic rather than oxidative metabolism (Seideman and Crouse, 1986; Gonzalez et al., 2009; Kellermeier et al., 2009; Garmyn et al., 2014; Kim, 2018). However, in swine, RAC supplementation increased the percentage of type IIB fibers over the original type IIA and IIX fibers (Aalhus et al., 1992; Gonzalez et al., 2009). In general, the relationship between βAA supplemented cattle and the increase observed in WBSF values could be attributed to the increased fiber diameter (Kellermeier et al., 2009).

Historically, some studies have reported that the supplementation of either ZH or RAC negatively impact tenderness (Avendaño-Reyes et al., 2006; Scramlin et al., 2010; Arp et al., 2013). Moreover, the supplementation of ZH is known to produce carcasses with reduced marbling scores, while most research examining RAC supplementation reported little to no effect on marbling score (Garmyn and Miller, 2014). While there are multiple studies demonstrating the benefit of using of hormonal implants and beta-adrenergic agonists in conjunction with one another (Platter et al., 2003; McPhee et al., 2006; Schneider et al., 2007), research has also reported negative effect of these compounds on carcass quality traits (Reiling and Johnson, 2003), decreased tenderness (Schneider et al., 2007), reduction in marbling scores, increased incidence of dark cutters (Roeber et al., 2000), and lower consumer taste panel scores (Barham et al., 2003).

Overall, steaks associated with βAA were rated as less desirable by consumers as well as by trained sensory panelists (Arp, 2012). It has been documented for both Ractopamine (Scramlin et al., 2010; Woerner et al., 2011; Boler et al., 2012) and Zilpaterol (Avendaño-Reyes et al., 2006; Garmyn et al., 2010; Scramlin et al., 2010) that the cattle are less tender (Consumer Union, 2013). Nonetheless, results from Scramlin et al. (2010) and Boler et al. (2012) indicated that the decrease in tenderness associated with Ractopamine could be overcome with postmortem aging.

Beta-adrenergic agonists, otherwise known as RAC and ZH are classified by their ability to bind and activate either the β1 or β2 receptor without being able to antagonize (i.e., blocking) the other. Unlike the βAA mentioned above, Lubabegron (Experior®, Elanco Animal Health; EX) is a new feed supplement that is able to activate the β3 receptor while also antagonizing β1 and β2 receptors. This specific capability allows EX to be classified as a β3- adrenergic agonist/antagonist (i.e., modulator). The β3 receptor is located within the fourth intracellular loop and is primarily found in adipose tissue (Fiems, 1987; Strosberg, 1992; Nisoli et al., 1996; Mersmann, 1998; Chikuni et al., 2008). On the other hand, β1 and β2 receptors are found in the third intracellular loop; thus allowing greater opportunities for theses receptors to become phosphorylated (Arch and Ainsworth, 1983; Strosberg, 1992; Emorine et al., 1994; Langin et al., 1995). Therefore, the β3 receptors location allows only limited desensitization compared to the other two subtypes (Mersmann, 1998). The objective of this study was to evaluate effects of the β3- adrenergic agonist/antagonist, Experior (EX), on postmortem lean color, muscle fiber typing, palatability and tenderness.

3.2 Materials and Methods

3.2.1 Live Animal Production and Fabrication Product Collection

Two thousand one hundred and sixty (2,160) British and European Continental crossbred steers were housed and fed at the Johnson Research Center in Northwestern Idaho. The experimental design was configured in a 4 x 3 factorial comprised of 2 factors; dose (0, 1.4, 3.2, 5.0 g/ton) and duration (28, 56, 84 d) in a complete randomized block design. Within each duration group, 15 pens (12 hd/pen) were assigned to each dosage level (0, 1.4, 3.2, 5.0 g/ton), resulting in 60 pens per duration and 180 total pens across all durations. Three blocks were assigned to each

study cycle, where each block consisted of 12 pens (12 hd/pen, 3 pens per treatment group) resulting in a total of 432 steers (36 pens) for each study cycle. Weights were taken and recorded one week and one day prior to the start of each treatment phase and final weights were collected at the end of the treatment phase. Additionally, all steers included in this study were never allowed to receive an implant or had to be explanted on or before day 99 (14 d prior to the start of the 84 d feeding duration group). All steers were fed a total mixed ration (TMR) including Monensin (40 g/ton) and Tylosin (8 g/ton) by day ~105 and were switched from a basal to a finishing ration at the start of the durational period (day ~85, day ~57, or day ~29) that would contain either 0, 1.4, 3.2 or 5.0 g/ton of Lubabegron (Experior®, Elanco Animal Health; EX) (100% dry matter basis).

Three carcasses that graded USDA Low Choice were selected in order to obtain a subset of carcasses. A total of 108 striploins were collected from the right side of each carcass during each kill cycle, resulting in a total of 540 striploins for further evaluation (carcass characteristics, color, muscle fiber typing, trained panel evaluation, tenderness). Data such as hot carcasses weight (HCW), *Longissimus* muscle area (LMA), adjusted fat thickness (aFT), and kidney pelvic and heart fat (KPH) were collected, were aFT was later converted to preliminary yield grade (PYG) and was used to calculate yield grade (YG). A 1.27-cm portion of the striploin was obtained from the striploin at the harvest facility to represent day 1, and the remaining striploins were labeled, vacuum packaged and shipped to the Center for Meat Quality and Safety at Colorado State University (CSU) under refrigeration. Upon arrival at CSU, striploins were fabricated into five 6.35-cm sections and randomly assigned to a postmortem (PM) aging period of 7, 14, 21 or 28 d. Once frozen, each 6.35-cm section was fabricated (in the frozen state) into two 2.54-cm steaks and one 1.27-cm steak, labeled, vacuum packed, and placed into frozen storage. The 2.54-cm steaks were randomly assigned to either trained sensory panel evaluations and or shear force analysis.

While all the aging periods were used for shear force analysis, trained sensory panel evaluation was performed only on steaks aged for 14 d due to the large sample number.

3.2.2 Instrumental Color

Objective lean color measurements were recorded immediately after grade data was collected. Measurements were obtained by trained Colorado State University personnel, using a portable spectrophotometer (Illuminant A, 6-mm aperture 10° observer; Hunter MiniScan XE, Hunter Labs, Reston, VA) that was calibrated before each use. A total of three readings of CIE L*- (lightness), a*-(redness), b*- values (yellowness) for each steak were collected and averaged for each carcass.

3.2.3 Muscle fiber typing

A total of 96 strip steaks (8/treatment) were immediately shipped post-grading to Kansas State University were Muscle Fiber Typing and Muscle fiber CSA evaluations were conducted. Methods used by Gonzalez et al (2007) and Gonzalez et al (2008) were followed for immunohistochemical staining with slight modifications. In order to block non-specific antigen sites, 5% horse serum was used in a phosphate-buffered saline (PBS) solution. In a primary antibody solution consisting of anti-myosin heavy chain type I (BAD.5, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA) hybridoma supernatant with an anti-α-dystrophin (1:50; Abcam, Cambridge, MA) cryosections were incubated for 60 min at room temperature. After the first PBS washing step, cryosections were incubated in rabbit anti-mouse AlexaFluor 568 (Invitrogen, San Diego, CA) and streptavidin AlexaFluor 488 (Invitrogen) for 45 min in order to detect dystrophin and MyHC type I. After the completion of the final PBS, Eclipse TE 2000-U microscope (Nikon, Lewisville, TX) equipped with an X-cite 120 epifluorescence

illumination system (EXFO, Mississauga, Ontario, Canada) was used in order to visualize the slides. Photomicrographs were captured using a Photometrics Cool Snap EF digital camera (Nikon) and analyzed for individual muscle fiber cross-sectional area and MyHC isoforms using the NIS-Elements software (Nikon). As described in Gonzalez et al. (2007, 2008), all fibers not labeled as MyHC type I were assumed to be Type II (IIA or IIX) fast-twitch.

3.2.4 Trained Sensory Panel

Strip loin steaks (n = 540) aged for 14 d were subjected to trained sensory panel evaluation. Steaks were randomly assigned to one of 45 panel sessions with 12 steaks evaluated per panel. Steaks were not only randomly assigned to a panel; they were randomly assigned a serving order within each panel. Prior to trained panel evaluations, panelists were trained to evaluate a multitude of flavor attributes (beef flavor, fat-like, brown roasted), off flavors (bloody serumy, metallic, oxidized, liver-like), basic tastes (sour, bitter, umami) as well as tenderness (initial, sustained, overall) and juiciness (initial, sustained, overall) on a fifteen point line scale with 0.5 increments according to the AMSA taste panel guidelines (Table 3.6). Each steak was evaluated by a trained sensory panel consisting of 6 qualified panelists with attribute training specifications being denoted in Table 3.6. Frozen steaks were tempered for 48– 60 h at 0 – 2°C in order to attain a raw internal temperature of 0 – 4°C at the time of cooking.

Before cooking, all excess external fat was trimmed off and weights were recorded in order to quantify cook loss. Cook loss was quantified (as percentage) by subtracting the initial weight from the final-cook weight, followed by the division of the initial-cook weight. Raw internal temperatures were measured using a calibrated, type K thermocouple thermometer (AccuTuff 340, model 34040, Cooper-Atkins Corporation, Middlefield, CT) that was placed in the geometric

center of each steak. Steaks were cooked in a combi-oven (Model SCC WE 61 E; Rational, Landsberg am Lech, Germany) until peak internal temperature of approximately 71°C was achieved. The type K thermocouple thermometer was placed in the geometric center of each steak to measure peak internal temperatures of cooked steaks.

Post-cooking, steaks were vacuum packaged and placed in a warm water bath at 55°C in order to maintain temperate throughout the panel. Cooked steaks were trimmed of all external fat and connective tissue and cut into 1-cm^2 cubed pieces and 2 – 3 pieces were served to each panelist for evaluation. Panelists rated each steak by entering a whole or half number into a blank 15 point line scale for the following attributes: initial and sustained tenderness, overall tenderness, initial and sustained juiciness, overall juiciness, beef flavor, fat-like, brown roasted, bloody/serumy, metallic, oxidized, liver-like, sour, bitter and umami. Each line scale indicated a very low presence on the left side and a very high presence of a specific attribute on the right.

3.2.5 Tenderness

3.2.5.1 Slice Shear Force

Striploin steaks that represented each postmortem aging period (1, 7, 14, 21, 28 d) were subjected to slice shear force (SSF) testing. Upon conclusion of aging, steaks were randomly assigned to a shear force cooking day. Within each shear force day, Warner-Bratzler shear force (WBSF) and SSF analysis was conducted. Before cooking, all excess external fat was trimmed off and the recording of pre- and post-cooking weights in order to quantify cook loss as well as pre- and post-cooking internal temperatures. Raw internal temperatures were measured using a calibrated, type K thermocouple thermometer (AccuTuff 340, model 34040, Cooper-Atkins Corporation, Middlefield, CT) that was placed in the geometric center of each steak. Steaks were

cooked in a combi-oven (Model SCC WE 61 E; Rational, Landsberg am Lech, Germany) until peak internal temperature of 71°C was achieved with a type K thermocouple thermometer inserted in the geometric center of each steak to measure peak internal temperatures during cooking.

Immediately post-cooking, weights were recorded and a 1-cm thick and 5-cm long slice were removed parallel to the longitudinal direction of the muscle fibers and sheared perpendicular to the muscle fibers, using the lateral portion (~1/3) of the strip steak. This was done by using a universal testing machine (Instron Corp., Canton, MA) equipped with a flat, blunt-end blade (crosshead speed: 500 mm/min, load capacity: 100 kg), resulting in the recording of a single SSF measurement for each steak.

3.2.5.2 Warner-Bratzler Shear Force

After SSF values were recorded, remaining portions of the steak were allowed to cool to room temperature (22°C) and an average of 6 cores (1.2-cm in diameter) were removed from each steak parallel to muscle fiber orientation. Each core was sheared once, perpendicular to the muscle fiber orientation, using a universal testing machine (Instron Corp., Canton, MA) fitted with a Warner-Bratzler shear head (crosshead speed: 200 mm/min, load cell capacity: 100 kg). Peak shear force was recorded and the values for each steak were averaged in order to obtain a single WBSF value for each steak.

3.2.6 Statistical Analysis

Separate mixed models were fit using multiple response variables, including those from carcass traits (HCW, aFT, KPH, LMA, YG, MS), color (L*, a*, b*, Hue, Chroma), muscle fiber type (Type I, II, IIA, IIX, ICSA, IIACSA, IIXCSA), tenderness (WBSF, SSF) and trained sensory panel ratings (cooking loss, initial tenderness and juiciness, sustained tenderness and juiciness,

overall tenderness, overall juiciness, beef flavor, umami, fat-like, bloody/serumy, brown roasted, metallic, oxidized, sour, liver-like and bitter). Fixed effects for carcass characteristics, color, and muscle fiber typing included dose (0, 1.4, 3.2, 5.0 g/ton; 100% dry matter) and feeding duration (28, 56, 84 d) plus the dose*feeding duration interaction. Pen was used as a random effect along with block being nested within cycle. Fixed effect of tenderness included dose (0, 1.4, 3.2, 5.0 g/ton; 100% dry matter), feeding duration (28, 56, 84 d) and postmortem aging periods (1, 7, 14, 21, 28 d) plus the dose*feeding and duration*aging interaction(s). Fixed effects for trained sensory panel included dose (0, 1.4, 3.2, 5.0 g/ton; 100% dry matter) and feeding duration (28, 56, 84 d) plus the dose*feeding duration interaction. Serve order was included as a separate fixed effect for all variables within the trained sensory panel analysis. Random effects for tenderness (stripID) and trained sensory panel (Session date, freeze date) were included in order to account for the designs. For each response, treatments were compared using Tukey adjusted (carcass characteristics, color, muscle fiber typing, tenderness) and Kenward-Roger adjusted (trained sensory panel) pairwise comparisons. All striploins missing a value or a score were removed from the final analysis to ensure consistency. Analysis was conducted using R Studio (RStudio 1.1.463) and the lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017) and emmeans (Lenth, 2019) packages with significance set at an alpha level of 0.05.

3.3 Results and Discussion

3.3.1 Subset carcass characteristics

Table 3.1 depicts carcass characteristics from the subset of carcasses that graded USDA Low Choice. The main effect of dose ($P < 0.05$) feeding duration (FD) was significant for HCW, LMA and YG; whereas, the interaction of dose x FD ($P < 0.05$) was significant for KPH (Table

3.2). Carcass weights improved ($P < 0.05$) with the supplementation of EX and as FD extended to 56 and 84 d when compared to non-supplemented cattle and cattle on feed for the last 28 d of the finishing period. Additionally, regardless of dose, EX produced larger LMAs with no difference in intermuscular fat such as aFT by any treatment combination of EX. The increase seen in HCW and LMA resulted in decreased yield grades when cattle were fed 3.2 and 5.0 g/ton, where those fed 1.4 produced YG that were similar to non-supplemented cattle.

3.3.2. Instrumental color

There was no dose (g/ton) x feeding duration (FD) interaction ($P > 0.05$) present for any color trait measured. The main effect of dose ($P < 0.05$) was significant for a* and b* values (Figure 3.2) while L* was not ($P > 0.05$). Striploins were subjected to color evaluation approximately 36 h postmortem, or postmortem aging day 1.

Effects of EX supplementation impacted redness (a*), yellowness (b*) chroma (color intensity) and hue angle (saturation). EX supplementation resulted in slightly lower redness and yellowness compared to controls as indicated by the lower a* ($P < 0.01$) and b* ($P < 0.01$) values, respectively. Previous studies, where both RAC and ZH were supplemented, b* values increased with RAC was supplementation (Avendaño-Reyes et al., 2006), whereas b* value was not affected by ZH supplementation (Avendaño-Reyes et al., 2006; Garmyn et al., 2014).

In the current study, L* value (lightness) did not differ between strip loins obtained from supplemented and non-supplemented cattle (Table 3.3). Literature has suggested that RAC (Quinn et al., 2008; Woerner et al., 2011) and ZH (Rogers et al., 2010) will not influence a* (redness), b* (yellowness) or L* (lightness) values. Both a* and b* values exhibited a 0.7 unit decrease when comparing lean from non-supplemented cattle to those fed 5.0 g/ton; therefore, as dose increased lean became less red.

Chroma and hue angle values are depicted in Table 3.3. Chroma is an important attribute in relation to fresh meat color and consumer acceptability. Striploins from non-supplemented cattle had a greater ($P < 0.01$) saturation (chroma) when compared to all EX treated cattle. The main effect of FD was significant ($P < 0.05$) for hue angle. Hue angle is considered the intensity of color which corresponds to the vividness of a specific color (Hunt and King, 2012). Increasing FD from 28 to 84 d decreased the saturation of the lean color by 0.5 units. Similarly, Martin et al. (2012) denoted that the addition of ZH to the finishing diet of feedlot steers resulted in steaks were less red (lower a* value) and less vivid (decreased saturation) than lean from both supplemented (RAC) and non-supplemented cattle. The initial indicator for consumer purchase acceptability is fresh lean color, with the perception that bright cherry red denotes freshness and quality. Although statistically significant, this difference may not correspond to a biological significance, indicating that there would not be a negative effect on consumer perception.

3.3.3 Muscle fiber typing

Muscle fiber types were measured using antibodies specific to myosin heavy chain type I, IIA and IIX isoforms. Immunoreactivity for all the muscle types were observed, suggesting that the *longissimus* muscle (LM) was composed of all three muscle fiber types (I, IIA, and IIX). The significance of least square means of the muscle fiber type IIX CSA among all treatment combination can be seen in Table 3.4. All percentage of muscle types from EX supplemented cattle were similar ($P > 0.05$) for all treatment combinations (Table 3.4). Previous studies have demonstrated that cattle supplemented with RAC show a shift from type I to IIA, or in other words, from those that rely on oxidative to glycolytic metabolism (Seideman and Crouse, 1986; Gonzalez et al., 2009; Kellermeier et al., 2009; Garmyn et al., 2014; Kim, 2018). Moreover, supplementation

with ZH resulted in an increase in the concentration of type IIX muscle fibers (Kellermeier et al., 2009; Baxa et al., 2010).

In the present study, Experior supplementation did not result in an any significant shifts among fiber type distribution. Therefore, in agreement with results of Baxa et al. (2010), the current study did not exhibit any difference in the concentration of type I or type IIA fibers. Rathmann et al. (2009) indicated that the type I and type IIX muscle fibers were not altered with the supplementation of ZH, though a decrease in the expression of type IIA was reported, much like the numerical shift observed in the current study for cattle supplemented with EX for the final 28 d of the finishing period. Smith et al. (1995) reported that βAAs express a greater response to type II muscle fibers; indicating that muscles that contain a greater amount of type II fibers will have a greater effectiveness to the βAA supplementation.

There was a two-way interaction between dose x feeding duration (FD) for the cross sectional area (CSA) of muscle fiber type IIX (Table 3.5); however, there were no other interactions present between any other CSA or proportions of muscle fibers types measured. Muscle fiber IIX CSA were similar ($P > 0.05$) across all feeding durations for muscles types produced from cattle fed 0, 1.4, and 3.2 g/ton of EX. Similar to the present study, type I and II muscle fiber CSA were not affected by the administration of RAC (Gonzalez et al., 2009). Type IIX CSA's from cattle supplemented at 1.4 and 3.2 g/ton DM increased numerically as FD increased, whereas it decreased ($P < 0.05$) in cattle supplemented at 5.0 g/ton with the smallest IIX CSA being from cattle that were fed the highest dose of EX for the longest duration. In regards to Type IIX CSA, our results were similar to those reported by Gonzalez et al. (2007), but the type IIX CSA's in the current study were much larger compared to those reported by Gonzalez et al. (2010), more than likely due to difference in dosages. Seideman and Crouse (1986) reported

muscles that contain larger muscle fibers similar to the *semitendinosus* have been proven to be less tender than those with smaller muscle fibers such as the *psoas major*. Consequently, the relative size of muscle fiber CSA in relation to tenderness will be discussed in later chapters.

As EX dosage increased from 3.2 and 5.0 g/ton for 84 d, the type IIX CSA's decreased ($P < 0.05$) from 5177 to 3907 μm. In general, as feeding duration increased, type IIX CSA numerically increased for control, 1.4, and 3.2 g/ton of EX supplementation, whereas with 5.0 g/ton the type IIX CSA decreased as FD increased. RAC supplemented at 200/mg/hd/d resulted in an increase in the percentage of type IIA fibers, while no effect was observed in either type I or type II muscle fiber CSA between controls or those fed RAC at 200 or 300 mg/hd/d (Gonzalez, 2008).

3.3.4. Trained Sensory Evaluation

Striploin steaks aged for 14 d postmortem (PM) were evaluated using trained sensory panelists and the mean values for attributes measured are presented in Table 3.7. Initial and sustained tenderness and juiciness values were averaged in order to obtain an overall score. No two-way interaction ($P > 0.05$) between dose x feeding duration (FD) was observed for any of the attributes measured. However, the main effect of dose and duration were significant ($P < 0.05$). Experior supplementation did not affect ($P > 0.05$) cook loss in striploin steaks among all treatment combinations. Similar results were reported by Boler et al. (2012) and Garmyn et al. (2014) when evaluating steaks from cattle supplemented with 200, 300 and 308 mg/hd/d, respectively.

Initial, sustained, overall tenderness, and initial and overall juiciness scores for steaks differed ($P < 0.05$) between control and EX supplemented cattle regardless of the FD. Steaks associated with EX supplementation were less tender and juicy than steaks from non-supplemented

cattle, although, the difference might be too small to contribute to a biological significance (Table 3.7). Similar results were reported by Gruber et al. (2008), Leheska et al. (2009), and Arp, (2012), with ZH supplemented cattle producing steaks that were less tender than those from non-supplemented cattle. Furthermore, (Arp, 2012) indicated that cattle supplemented with 200 mg/hd/d of RAC produced steaks that were similar in tenderness to that of the control. Conversely, at 14 d of PM aging βAA supplementation resulted in decreased tenderness (Rodas-González et al., 2012; Bloomberg et al., 2013). There was no difference ($P > 0.05$) in juiciness scores with FD in the current study. Additionally, Leheska et al. (2009) indicated that βAA supplementation did not affect overall juiciness scores when FD extended from 20 to 40 d for steers or heifers. On the other hand, Garmyn et al. (2014) reported that ZH decreased sustained juiciness scores; whereas, initial juiciness was not affected. Similarly, Arp (2012) indicated that ZH decreased juiciness scores compared to controls, whereas RAC did not influence juiciness scores.

There was no dose x FD interaction for any off-flavor or the other basic taste attributes evaluated. However, the main effect of FD was significant ($P < 0.05$) for the off flavor, liver-like and bitterness. When FD increased from 28 to 56 and 56 to 84 d, panelists were less likely to detect the presence of liver-like. Additionally, bitterness increased ($P < 0.05$) by 0.8 units as FD extended from 28 and 56 when compared to 84 d. Gruber et al. (2008) and Leheska et al. (2009) reported that βAA supplementation decreased the prevalence of beef flavor in heifers as well as flavor intensity in both steers and heifers. However, in agreement with the current study, Arp (2012) reported no difference in beef flavor with βAA supplementation. Moreover, no differences ($P < 0.05$) in any other flavor attributes were reported in the current study. Overall, EX supplementation had minimal impact on the flavor evaluated using trained panelists after 14 days of aging.

3.3.5 Tenderness

3.3.5.1 Slice Shear Force

There was no three-way interaction ($P = 0.21$) between dose x feeding duration (FD) x postmortem (PM) aging as well as no two-way interaction ($P = 0.12$) between dose x FD for slice shear force (SSF) values. However, the dose x postmortem aging interaction was significant ($P < 0.05$; Table 3.8), as well as the main effect ($P < 0.05$) of FD. As feeding duration extended from 28 to 84 d, the SSF values increased ($P < 0.05$; Figure 3.4); however, when cattle were fed EX for 56 d, resulting steaks had SSF values that were similar ($P > 0.05$) to those fed for 28 and 84 d. A decrease ($P < 0.05$) in SSF values was observed after 7 d of aging for both non-supplemented (4.8 kg) and supplemented (5.6, 6.2, and 5.3 kg respectively for 28, 56, and 84 d FD) strip steaks (Table 3.8). Once steaks were aged for 14 d, all steaks associated with EX supplementation (15.3, 16.3 and 15.8 kg) had similar ($P > 0.05$) SSF whereas the controls produced steaks that were more ($P < 0.05$) tender (13.5 kg). By 21 d of aging, all steaks from supplemented cattle had similar ($P > 0.05$) tenderness values but were tougher ($P < 0.05$) than controls. However, once 28 d of aging, all steaks from supplemented and non-supplemented cattle resulted in similar ($P > 0.05$) SSF values. Garmyn et al. (2014) reported that steaks from cattle supplemented with ZH were tougher than those fed either RAC or a control diet at 14 and 21 d of aging. Garmyn et al. (2014) also observed that increasing PM aging from 14 to 21 d improved the proportion of steaks eligible to be labeled as Certified tender and very tender.

As described by ASTM (2011), a tenderness marketing claim is available for labeling and advertisements associated with beef cuts in order to promote and distinguish a premium marketplace. Throughout the years, the beef industry and the United States Department of Agriculture have established thresholds for tenderness by considering categories such as very

tender (WBSF < 3.9 kg; SSF < 15.4 kg; ASTM, 2011) and tender (WBSF 4.4 kg; SSF 20.0 kg; ASTM, 2011). If a SSF value is less than 20.0 kg, the product is eligible to enter a "Guaranteed Tender" program (ASTM, 2011). In the current study, once 7 and 14 d of PM aging was reached all treatment dosages were tender (15.3 – 20 kg), whereas the majority of steaks aged for 21 d would be eligible for a "Certified Very Tender"(< 15.3 kg) label claim. With the average PM fabrication time for majority of beef cuts at the retail level being between 25.9 d (Henderson, 2016), it was clear that, regardless of EX supplementation, steaks produced would be considered tender and very tender.

The interaction of means for all treatment combinations including PM aging periods for SSF and WBSF are presented in Table 3.5. Control steaks and those produced from cattle that were fed 3.2 g/ton, were tender ($P < 0.05$) compared to all the doses on day 1 of aging. At 28 d of aging, all steaks regardless of treatment had similar SSF ($P < 0.05$; Table 3.5).

3.3.5.2 Warner Bratzler Shear Force

There was no three-way interactions ($P = 0.91$) between dose x feeding duration (FD) x post-mortem (PM) aging or two-way interactions between dose x FD for Warner-Bratzler shear force (WBSF) suggesting that the treatments elicit similar effects ($P = 0.71$). The dose x PM aging interaction was significant ($P < 0.05$) and is presented in Table 3.9.

The main effect of feeding duration for WBSF was significant ($P < 0.05$). Warner-Bratzler shear force values increased ($P < 0.05$) by 0.26 kg when cattle were fed for the final 84 d compared to 28 d (Figure 3.10). However, WBSF values were similar ($P > 0.05$) for steaks from cattle fed for 28 vs. 56 d and 56 vs. 84 d. Therefore, the only difference was between the two extreme feeding duration. While the increase may have been statistically significant, there may not have been a

substantial difference biologically in tenderness between the FD as consumers are able to detect difference in tenderness only when WBSF values differed by 0.5 kg (Miller et al., 1995a).

Table 3.9 demonstrates that control steaks and those produced from steers fed 1.4 g/ton resulted in similar ($P > 0.05$) WBSF values; however, were more ($P < 0.05$) tender than those from steers fed 3.2 and 5.0 g/ton with only 1 d of postmortem aging. Scramlin et al. (2010) indicated that WBSF values from steers fed ZH at 75 mg/hd/d were 6.89, 6.30 5.28 and 4.29 kg at postmortem aging days 3, 7, 14, and 21 respectively. In the current study, at the medium dose of EX (3.2 g/ton), WBSF values were5.12, 3.74, 3.45 and 3.12 kg when aged for 0, 7, 14, and 21 d (Table 3.9). Scramlin et al. (2010) also reported that when RAC was supplemented at 200 mg/hd/d WBSF values of 5.36, 4.63, 3,78, and 3.28 kg were denoted at aging days of 3, 7, 14, and 21 d, indicating that the tenderness improved with aging. When cattle were supplemented with 8.33 mg/kg of ZH for the final 20, 30, and 40 d, WBSF values decreased by 0.89, 1.06, and 1.15. kg, respectively, whereas, within the control steaks a decrease of only 0.42 kg was observed as postmortem aging time increased from 7 to 21 d (Rathmann et al., 2009). A subsequent study by Rathmann et al. (2012) examined effects of ZH (8.33 mg/kg) supplementation on heifers when fed for the final 20 d of the finishing period and reported that, as postmortem aging times increased (7, 14, 21 d), ZH treated steaks were tougher than the controls. On the other hand, Van Donkersgoed et al. (2011) reported no differences in tenderness in heifers regardless of the βAA administration. In the present study, on average steaks, became more tender as each postmortem aging time point was achieved (Table 3.9).

In the current study, when 7 d PM aging was reached, all supplemental doses produced similar ($P < 0.05$) tenderness values compared to steaks from carcasses of cattle treated as negative controls (Table 3.9). Quinn et al. (2008) reported similar shear force values for control LM steaks

aged for 14 d and those supplemented with 200 mg/hd/d of RAC for 28 d of the finishing period. Boler et al. (2012) reported similar WBSF values between striploins aged for 7, 14 and 21d when steers were fed 200mg/hd/d RAC when compared with steers that were fed a control diet. However, both striploin steaks from RAC (200 mg/hd/d) and control fed cattle were more tender than steaks produced from steers fed 300 mg/hd/d (Boler et al., 2012). When ZH was fed in a conventional implant system, WBSF values decreased from 5.43 kg on 7 d to 4.50 kg on 14 d, with no further improvement observed with 21 d of aging (4.52 kg; Kellermeier et al., 2009). Moreover, when RAC was supplemented at 312 mg/hd/d to market dairy cows, the resulting steaks were subjected to a 14 d postmortem aging time prior to tenderness evaluation, where no differences in WBSF values were reported (Allen et al., 2009).

All steaks from supplemented and non-supplemented cattle aged for 28 d had lower ($P < 0.05$) shear force values than those aged for 1, 7, and 14 d (Table 3.9). Previous research indicated that RAC supplementation at 200 mg/hd/d for the final 28 d of the finishing period resulted in LM steaks that were tougher than controls (Gruber et al., 2008). Allen et al. (2009) reported that RAC supplementation at 312 mg/hd/d for 28 d increased WBSF values compared to the control (4.51 vs. 4.32 kg). Garmyn et al. (2014) reported a decrease of 0.33 and 0.29 kg in WBSF when RAC steaks were aged for 14 and 21 d. As expected, overall, the WBSF values for the steaks consistently decreased (or became more tender) as postmortem aging days increased in the current study. Woerner et al. (2011) reported contradictory results that RAC supplementation on LM tenderness was not affected by postmortem aging. Nevertheless, studies have indicated that when subjected to PM aging of 14 d or greater, the negative influence on tenderness associated with the feeding of βAA's are greatly decreased (Scramlin et al., 2010; Boler et al., 2012).

Use of extended aging periods has been historically used across the beef industry as a method of enhancing tenderness. Figure 3.3 illustrates that when averaged over all postmortem aging periods, WBSF increased ($P < 0.05$) as FD increased (28, 56, 84 d). Moreover, as the FD increased, the percentage of "Very Tender" steaks decreased to 72.3, 69.8, and 65.7 percent, respectively. Additionally, Figure 3.4 illustrates steaks categorized as very tender (< 3.9 kg; ASTM, 2011), tender (3.9 – 4.4 kg; ASTM, 2011) or tough (> 4.4 kg; ASTM, 2011) depending on their associated WBSF values for all the treatment combinations and postmortem aging periods. Steaks from carcasses of steers that were fed for the final 28 d of the finishing period produced the highest of percentage (83.03%) of steaks that would be eligible for either a "Certified Very Tender" or "Tender" label claim, while also producing the lowest percentage (16.90%) of tough steaks. The percentages of very tender and tender steaks decreased by 2.38% (to 80.65%) and 5.28% (to 77.75%) for those fed for 56 and 84 d. Thus, the frequency of steaks shearing greater than 4.4 kg (tough) increased when cattle were fed EX for 56 (19.35%) and 84 d (22.28%) compared to those fed for the final 28 d of the finishing period (16.90%). Experimental design of the current study permitted examination of postmortem aging effects on LM utilizing WBSF and SSF between supplemented and control steaks throughout different durations of supplementation as presented in Table 3.10.

3.3.6 Relationship between WBSF and SSF

Figure 3.5 illustrates the correlation between Warner-Bratzler shear force (WBSF) and Slice shear force (SSF). A Pearson's correlation analysis was performed to compare WBSF and SSF and results demonstrated that a positive correlation (r = 0.64) between the two existed, indicating a moderately strong relationship. Previous studies by Derington et al. (2011) reported only a positive correlation of 0.52 between the two, whereas Shackelford et al. (1999) reported a

much stronger correlation of 0.80 between WBSF and SSF. Moreover, Lorenzen et al. (2010) reported an r-value of 0.61 when comparing SSF and WBSF. Overall, both SSF and WBSF followed a similar trend in response to aging. Figure 3.6 illustrates the comparison of least squares means along five PM aging periods with two different shear force methods (WBSF and SSF). Both shear force methods exhibited a linear reduction in mean values when aged over a 28-d period. Over the course of a PM aging periods, SSF (7.89 kg) showed a greater numerical decline of mean values over WBSF (1.93 kg). Regardless, except for those that were aged for 0 d, the majority of WBSF and SSF values fell within the acceptability threshold for consumer acceptance and will as qualify for certified tender programs once 7 d of aging was achieved.

3.4 Conclusion

The carcass data from the subset of low choice carcasses indicated that Experior supplemented cattle had heavier carcass weights and larger *longissimus* muscle areas compared to non-supplemented cattle as well as no changes in adjusted fat thickness or kidney pelvic and heart fat percentage; therefore, resulting in carcasses with lower yield grades. Additionally, no detrimental shifts were observed for muscle fiber type percentages and there was no differences in type I and IIA cross sectional area. However, when the high dose (5.0 g/ton) of EX was supplemented and feeding durations extended type IIX cross sectional area decreased. Results of the sensory analysis indicated that the non-supplemented cattle produced strip steaks that were slightly juicier and more tender than those from EX supplemented cattle regardless of dose, whereas no difference was observed in relation to feeding durations from carcasses that graded USDA Low Choice. Although there may have been some statistical differences in flavor between steaks from supplemented and non-supplemented cattle, there were no major differences in positive or negative flavor attributes indicating that EX did not alter the flavor profile of the meat

and would not affect the consumer acceptability. All steaks (supplemented and non-supplemented) that were subjected to a minimum 7 d of PM aging would be eligible for a “certified very tender” label. Although the control steaks had lower shear force values than the treatments, there was no difference in tenderness after 21 d of aging. Overall, EX supplementation did not have any considerable implications on the flavor and tenderness of beef strip loin steaks.

Table 3.1. Effects of feeding 4 doses of Experior (EX; 0, 1.4, 3.2, 5.0 g/ton DM basis) for 3 feeding durations (28, 56, 84 d) on carcass characteristics of subset carcasses of feedlot steers that graded USDA Low Choice (n = 538).

Trait	Experior, g/ton dry matter (DM)				P-value	Feeding duration (FD)			P-value	SEM[1]
	0	1.4	3.2	5.0		28	56	84		
HCW, kg[2]	392.64[b]	399.79[ab]	401.28[a]	399.91[ab]	0.04	393.88[b]	399.74[ab]	401.86[a]	0.02	2.62
Adjusted fat thickness, in	0.55	0.58	0.55	0.57	0.68	0.58	0.54	0.56	0.22	0.01
LM area, cm2[4]	90.90[b]	93.22[ab]	95.16[a]	93.41[ab]	< 0.01	92.39	93.55	93.55	0.36	0.06
Marbling score[3]	488.30	482.53	477.71	470.81	0.16	480.32	481.01	478.86	0.95	2.87
USDA YG	338[a]3.38[a]	3.25[ab]	3.17[b]	3.19[b]	0.03	3.31	3.18	3.27	0.15	0.03

[ab] Within row, values with different superscripts are different (P < 0.05)

[1]Standard error of the means

[2] HCW - hot carcass weight

[3] Marbling scores 300 = slight; 400 = small; 500 = modest

[4] LM area- longissimus muscle area

[5] KPH% - Percent Kidney Pelvic and Heart fat

Table 3.2. Least square means of kidney pelvic and heart fat (KPH) by the interaction of dose X feeding duration from beef from a subset of carcasses from cattle fed 4 doses)of Experior (EX; 0, 1.4, 3.2, 5.0 g/ton on DM basis) for 3 feeding durations (28, 56, 84 d) (n = 538).

Dose (g/ton)[2]	Feeding duration (FD)[3]			SEM[1]
	28	56	84	
0	1.94^{xy}	1.87	1.96	0.084
1.4	1.95^{xy}	2.05	2.15	
3.2	1.80^{b,y}	1.96^{ab}	2.19^{a}	
5.0	2.12^{x}	1.94	1.99	

[1]Pooled standard error of the mean

^{abcd} Within row, values with different superscript are different ($P < 0.05$)

^{xyz} Within column, values with different superscript are different ($P < 0.05$)

Table 3.3. Least square means of Hue angle and chroma values of beef longissimus muscle by effects of feeding cattle 4 doses of Experior (EX; 0, 1.4, 3.2, 5.0 g/ton on DM basis) for 3 feeding durations (28, 56, 84 d) (n = 540) from a subset of carcasses grading USDA Low Choice.

Trait	Experior, g/ton dry matter (DM)				P-value	SEM[1]	Feeding duration (FD)			P-value	SEM[1]
	0	1.4	3.2	5.0			28	56	84		
L*	39.9	39.4	39.3	39.2	0.175	0.79	39.4	39.5	39.4	0.853	0.79
Hue	52.8	53.1	53.0	53.0	0.521	0.23	53.3[a]	52.9[ab]	52.8[b]	0.02	0.22
Chroma	23.9[a]	23.5[ab]	23.1[b]	23.0[b]	<0.01	0.33	23.5	23.3	23.3	0.64	0.31

[ab] Within row, values with different superscripts are different ($P < 0.05$)

[1] Hue angle – The of saturation

[2] Chroma – color intensity

Table 3.4. Least square means of muscle fiber type (%) and cross-sectional area (CSA) of beef longissimus muscle by effects of feeding cattle 4 doses of Experior (EX; 0, 1.4, 3.2, 5.0 g/ton on DM basis) for 3 feeding durations (28, 56, 84 d) (n = 96) from a subset of carcasses grading USDA Low Choice.

Type	Experior, g/ton dry matter (Dose)				SEM[1]	*P*-value	Feeding duration (FD)			SEM	*P*-value	Interaction	
	0	1.4	3.2	5			28	56	84			SEM	P- value
ICSA, μm^2	2417	2411	2509	2400	115	0.902	2334	2432	2536	99.30	0.362	199	0.059
IIACSA, μm^3	3486	3401	3660	3328	193	0.356	3497	3385	3524	118	0.680	237	0.129
Type I, %[4]	29.9	29.8	29.8	29.2	1.00	0.970	30.3	30.0	28.7	0.87	0.381	1.74	0.129
Type IIA, %[5]	35.1	35.0	34.0	36.0	1.22	0.732	35.4	34.8	34.9	1.05	0.920	2.11	0.326
Type IIX, %[6]	35.0	35.3	36.2	34.8	1.27	0.876	34.3	35.2	36.4	1.10	0.403	2.20	0.597

[abc] Within row, values with different superscript are different ($P < 0.05$).

[1]Pooled standard error of the mean

[2]Type I muscle fiber cross sectional area

[3]Type IIA muscle fiber cross sectional area

[4]Type I muscle fiber

[5]Type IIA muscle fiber

[6]Type IIX muscle fiber

Table 3.5. Least square means of muscle fiber type IIX Cross Sectional Area (IIXCSA) by the interaction of dose X feeding duration from beef longissimus muscle obtained from cattle fed 4 doses of Experior (EX; 0, 1.4, 3.2, 5.0 g/ton on DM basis) for 3 feeding durations (28, 56, 84 d) (n = 96) from a subset of carcasses grading USDA Low Choice.

Dose (g/ton)[2]	Feeding duration (FD)[3]			SEM[1]
	28	56	84	
0	4522[a,x]	4513[a,x]	4917[a,xy]	338
1.4	4396[a,x]	4650[a,x]	5100[a,xy]	
3.2	4931[a,x]	5071[a,x]	5177[a,x]	
5.0	5516[a,x]	4168[b,x]	3907[b,y]	

[1]Pooled standard error of the mean
[abcd] Within row, values with different superscript are different ($P < 0.05$)
[xyz] Within column, values with different superscript are different ($P < 0.05$)

Table 3.6. Definitions and references for beef flavor attributes and intensities.

Attribute	Definition	Reference
Beef flavor	Amount of beef flavor identity in the sample	Swanson® Beef Broth = 5.0 (aroma and flavor) 80% lean ground beef = 7.0 (aroma and flavor) Beef brisket = 11.0 (aroma and flavor)
Fat-like	Aromatics associated with cooked animal fat	
Brown roasted	A round, full aromatic generally associated with beef suet that has been broiled	Beef suet = 8.0 (aroma and flavor) 80% lean ground beef = 10.0 (aroma and flavor)
Bloody/serumy	Aromatics associated with blood on cooked meat products; closely related to metallic aromatic	USDA Choice strip steak = 5.5 (aroma and flavor) Beef brisket = 6.0 (aroma and flavor)
Metallic	The impression of slightly oxidized metal, such as iron, copper, and silver spoons	0.10% potassium chloride solution = 1.5 (flavor) USDA choice strip steak = 4.0 (aroma and flavor) Dole® canned pineapple juice = 6.0 (aroma and flavor)
Oxidized	Aromatic commonly associated with oxidized fat and oils. These aromatics may include cardboard, painty, varnish, and fishy.	Microwaved Wesson® vegetable oil (3 min at high) = 7.0 (flavor) Microwaved Wesson® vegetable oil (5 min at high) = 9.0 (flavor)
Liver-like	Aromatics associated with cooked organ meat/liver	Beef liver = 7.5 (aroma and flavor) Braunschweiger liver sausage = 10.0 (aroma and Flavor—must taste and swallow)
Sour	Fundamental taste factor associated with citric acid	0.015% citric acid solution = 1.5 (flavor) 0.050% citric acid solution = 3.5 (flavor)
Bitter	The fundamental taste factor associated with a caffeine solution	0.01% Caffeine Solution = 2.0 0.02% Caffeine Solution = 3.5
Umami	Flat, salty, somewhat brothy; taste of glutamate, salts of amino acids, and other molecules called nucleotides	0.035% accent flavor enhancer solution = 7.5 (flavor)
Flavor Intensities	Universal scale for flavor intensities	2.0 - Soda flavor in saltine crackers 5.0 - Apple flavor in Motts apple sauce 7.0 - Orange flavor in Minute maid orange juice 10.0 - Grape flavor in Welch's grape juice 12.0 - Cinnamon flavor in Big red chewing gum
Tenderness		5. 0 – Cross cut beef shank 180°F 6.0 – Select Strip steak 178°F 9.0 – Eye of round 160°F 14.0 – Tenderloin 150°F
Juiciness		2. 0 - Carrot 8.0 - Cucumber 10.0 – Apple 15.0 - Watermelon

15 point line scale was used

1 * 2 * 3 * 4 * 5 * 6 * 7 * 8 * 9 * 10 * 11 * 12 * 13 * 14 * 15

Slight | Moderate | Strong

0 – none	5 –	11 –
1 –	6 – Slightly intense	12 – very intense
2 – Barely detectable	7 –	13 –
3 –	8 – moderately intense	14 –
4 – Identifiable, not very intense	9 –	15 – extremely intense
	10 – intense	

Table 3.7. Effects of feeding 4 doses of Experior (EX; 0, 1.4, 3.2, 5.0 g/ton DM basis) for 3 feeding durations (28, 56, 84 d) on cooking loss and trained sensory scores of beef longissimus muscle subjected to 14 days of aging (n= 535) from a subset of carcasses grading USDA Low Choice.

Item	Experior, g/ton dry matter (DM)				*P*-value	SEM[1]	Feeding Duration (FD)			*P*-value	SEM
	0	1.4	3.2	5.0			28	56	84		
Cooking loss[2]	0.22	0.22	0.21	0.23	0.09	0.01	0.22	0.22	0.22	0.94	0.01
Initial tenderness[3]	10.25[a]	9.93[b]	9.90[b]	9.80[b]	< 0.01	0.12	10.04	9.88	9.90	0.18	0.12
Sustained tenderness[3]	10.21[a]	9.90[b]	9.80[b]	9.70[b]	< 0.01	0.13	9.99	9.81	9.81	0.12	0.12
Initial juiciness[3]	8.27[a]	8.09[b]	8.10[b]	8.10[b]	0.04	0.10	8.18	8.10	8.15	0.51	0.10
Sustained juiciness[3]	8.33	8.10	8.17	8.14	0.06	0.11	8.25	8.16	8.19	0.42	0.10
Overall tenderness[3]	10.23[a]	9.90[b]	9.85[b]	9.73[b]	< 0.01	0.12	10.02	9.85	9.86	0.14	0.12
Overall juiciness[3]	8.30[a]	8.12[b]	8.14[b]	8.12[b]	0.04	0.10	8.21	8.13	8.17	0.46	0.10
Beef flavor[3]	7.97	8.05	7.98	7.94	0.31	0.09	8.01	7.96	7.99	0.58	0.08
Fat-like[3]	1.97	1.92	1.92	1.85	0.06	0.05	1.93	1.92	1.89	0.57	0.04
Brown roasted[3]	6.74	6.84	6.68	6.72	0.26	0.14	6.79	6.69	6.74	0.38	0.13
Bloody serumy[3]	1.11	1.06	1.10	1.04	0.42	0.06	1.08	1.09	1.07	0.87	0.06
Metallic[3]	1.49	1.49	1.49	1.55	0.35	0.05	1.52	1.50	1.50	0.79	0.04
Oxidized[3]	0.88	0.90	0.83	0.85	0.38	0.07	0.88	0.86	0.86	0.79	0.07
Liver-like[4]	0.58	0.50	0.49	0.50	0.10	0.05	0.57[a]	0.51[ab]	0.47[b]	0.02	0.04
Sour[3]	1.27	1.30	1.27	1.29	0.63	0.06	1.27	1.28	1.28	0.99	0.05
Bitter[3]	0.80	0.83	0.83	0.83	0.82	0.05	0.80[b]	0.80[b]	0.88[a]	< 0.01	0.04
Umami[3]	1.06	1.09	1.12	1.04	0.22	0.07	1.08	1.07	1.08	0.88	0.07

[ab] Within row, values with different superscripts are different (P < 0.05)

[1]Standard error of the mean

[2]Individual strip steaks were weighed prior and post cooking in order to determine the cooking loss percentage

[3]0 = none; 8 = moderately intense; 15 = extremely intense

[4]0 = none, 5 = moderately intense; 10 = extremely intense

Table 3.8. Least square means of Slice Shear Force (SSF; kg) values by the interaction of dose X days from beef longissimus muscle obtained from a subset of cattle grading USDA Low Choice fed 4 doses of Experior (EX; 0, 1.4, 3.2, 5.0 g/ton DM basis) and subjected to 5 postmortem aging periods (1, 7, 14, 21, 28 d).

Dose (g/ton)	Postmortem (PM) aging day					SEM[1]
	0	7	14	21	28	
0	$19.6^{a,z}$	$14.8^{b,y}$	$13.5^{c,y}$	$13.4^{c,y}$	$13.2^{c,x}$	0.41
1.4	$21.4^{a,y}$	$15.8^{b,y}$	$15.3^{bc,x}$	$14.3^{cd,xy}$	$13.4^{d,x}$	0.42
3.2	$22.4^{a,xy}$	$16.2^{b,y}$	$16.3^{b,x}$	$14.1^{c,xy}$	$14.6^{c,x}$	0.42
5.0	$23.2^{a,x}$	$17.9^{b,x}$	$15.8^{c,x}$	$15.5^{c,x}$	$13.7^{d,x}$	0.44

[1] Pooled standard error of the means

[abcd] Within row, values with different superscript are different ($P < 0.05$)

[xyz] Within column, values with different superscript are different ($P < 0.05$)

Table 3.9. Least square means of Warner-Bratzler Shear Force (WBSF; kg) values by the interaction of dose X days from beef longissimus muscle obtained from a subset of cattle grading USDA Low Choice fed 4 doses of Experior (EX; 0, 1.4, 3.2, 5.0 g/ton DM basis) and subjected to 5 postmortem aging periods (1, 7, 14, 21, 28 d).

Dose (g/ton)	Postmortem (PM) aging day					SEM[1]
	0	7	14	21	28	
0	$4.48^{a,y}$	$3.15^{b,y}$	$3.02^{bc,y}$	$2.86^{cd,y}$	$2.75^{d,y}$	0.077
1.4	$4.72^{a,y}$	$3.52^{b,x}$	$3.21^{c,xy}$	$3.18^{c,x}$	$2.83^{d,xy}$	0.077
3.2	$5.12^{a,x}$	$3.74^{b,x}$	$3.45^{c,x}$	$3.12^{d,xy}$	$3.07^{d,x}$	0.078
5.0	$5.02^{a,x}$	$3.77^{b,x}$	$3.44^{c,x}$	$3.26^{cd,x}$	$3.08^{d,x}$	0.080

[1]Pooled standard error of the means

[abcd] Within row, values with different superscript are different ($P < 0.05$)

[xyz] Within column, values with different superscript are different ($P < 0.05$)

Table 3.10. Effects of feeding 4 doses of Experior (EX; 0, 1.4, 3.2, 5.0 g/ton DM basis) for 3 feeding durations (28, 56, 84 d) on Warner-Bratzler (WBSF) (n = 2659) and Slice shear force (SSF) values of longissimus muscles after 1, 7, 14, 21, and 28 d of postmortem aging (n = 2764) from a subset of carcasses grading USDA Low Choice.

Postmortem aging day	Feeding duration (FD)	No Experior 0 g/ton	Experior at 1.4 g/ton	Experior at 3.2 g/ton	Experior at 5.0 g/ton	SEM
SSF, kg						
0	28	$19.59^{a,y}$	$20.25^{a,y}$	$21.68^{a,x}$	$21.90^{a,x}$	0.362
	56	$19.59^{b,y}$	$21.38^{ab,x}$	$22.06^{ab,x}$	$23.08^{a,w}$	0.360
	84	$19.77^{b,y}$	$22.45^{a,x}$	$23.53^{a,x}$	$24.58^{a,w}$	0.362
7	28	$15.45^{a,z}$	$14.62^{a,z}$	$15.16^{a,y}$	$17.05^{a,y}$	0.361
	56	$14.28^{b,z}$	$16.13^{ab,yz}$	$17.76^{a,y}$	$17.71^{a,x}$	0.360
	84	$14.66^{b,z}$	$16.78^{ab,y}$	$15.79^{b,yz}$	$18.92^{a,x}$	0.363
14	28	$13.95^{a,z}$	$14.67^{a,z}$	$14.88^{a,y}$	$14.88^{a,yz}$	0.363
	56	$13.36^{b,z}$	$16.79^{a,y}$	$16.54^{a,yz}$	$16.51^{a,xy}$	0.360
	84	$13.14^{c,z}$	$14.31^{bc,z}$	$17.51^{a,y}$	$16.19^{ab,yz}$	0.362
21	28	$14.48^{ab,z}$	$13.71^{ab,z}$	$12.64^{b,z}$	$15.30^{a,yz}$	0.362
	56	$12.64^{b,z}$	$14.38^{ab,z}$	$15.25^{a,z}$	$14.46^{ab,yz}$	0.36
	84	$12.97^{b,z}$	$14.96^{ab,yz}$	$14.39^{ab,z}$	$16.64^{a,xy}$	0.362
28	28	$13.55^{a,z}$	$13.16^{a,z}$	$14.12^{a,yz}$	$13.23^{a,z}$	0.363
	56	$13.03^{a,z}$	$13.89^{a,z}$	$15.08^{a,z}$	$13.86^{a,z}$	0.360
	84	$13.01^{a,z}$	$13.24^{a,z}$	$14.63^{a,z}$	$14.14^{a,z}$	0.362
WBSF, kg						
0	28	$4.58^{ab,x}$	$4.40^{b,x}$	$4.95^{a,x}$	$4.86^{ab,x}$	
	56	$4.43^{b,y}$	$4.87^{ab,x}$	$5.13^{a,x}$	$4.92^{ab,w}$	0.067
	84	$4.44^{b,y}$	$4.89^{ab,w}$	$5.26^{a,x}$	$5.29^{a,x}$	
7	28	$3.29^{a,y}$	$3.25^{a,y}$	$3.56^{a,y}$	$3.59^{a,y}$	0.068
	56	$3.09^{b,z}$	$3.53^{ab,y}$	$3.82^{a,y}$	$3.75^{a,x}$	0.067
	84	$3.07^{b,z}$	$3.79^{a,x}$	$3.83^{a,y}$	$3.99^{a,y}$	0.067
14	28	$3.09^{a,yz}$	$2.95^{a,yz}$	$3.31^{a,yz}$	$3.12^{a,z}$	0.068
	56	$3.06^{b,z}$	$3.31^{ab,y}$	$3.43^{ab,yz}$	$3.56^{a,yx}$	0.067
	84	$2.91^{b,z}$	$3.37^{ab,y}$	$3.62^{a,yz}$	$3.65^{a,yz}$	0.068
21	28	$2.94^{a,yz}$	$3.00^{a,yz}$	$3.02^{a,z}$	$3.14^{a,yz}$	0.068
	56	$2.78^{a,z}$	$3.20^{a,yz}$	$3.04^{a,z}$	$3.17^{a,yz}$	0.067
	84	$2.87^{b,z}$	$3.34^{ab,y}$	$3.31^{ab,z}$	$3.47^{a,z}$	0.068
28	28	$2.72^{a,z}$	$2.78^{a,z}$	$2.91^{a,z}$	$2.79^{a,z}$	0.068
	56	$2.75^{a,z}$	$2.86^{a,z}$	$3.10^{a,z}$	$3.00^{a,z}$	0.067
	84	$2.78^{b,z}$	$2.84^{b,z}$	$3.20^{ab,z}$	$3.46^{a,z}$	0.068

[1]Pooled standard error of the means

[abc]Within row, values with different superscript are different ($P < 0.05$)

[wxyz]Within column, values with different superscripts are different ($P < 0.05$)

Age day 1 6.35-cm			Age day 7 6.35-cm			Age day 14 6.35-cm			Age day 21 6.35-cm			Age day 28 6.35-cm		
Shear Force Analysis		Proteomics	Shear Force Analysis			Shear Force Analysis	Trained Sensory Evaluation		Shear Force Analysis			Shear Force Analysis		
2.54cm	2.54cm	1.27	2.54cm	2.54cm	1.27	2.54cm	2.54cm	1.27	2.54cm	2.54cm	1.27	2.54cm	2.54	1.27

Figure 3.1. Schematic of striploin breakdown for aging and quality evaluation. The sections were randomly allocated to each of the aging period.

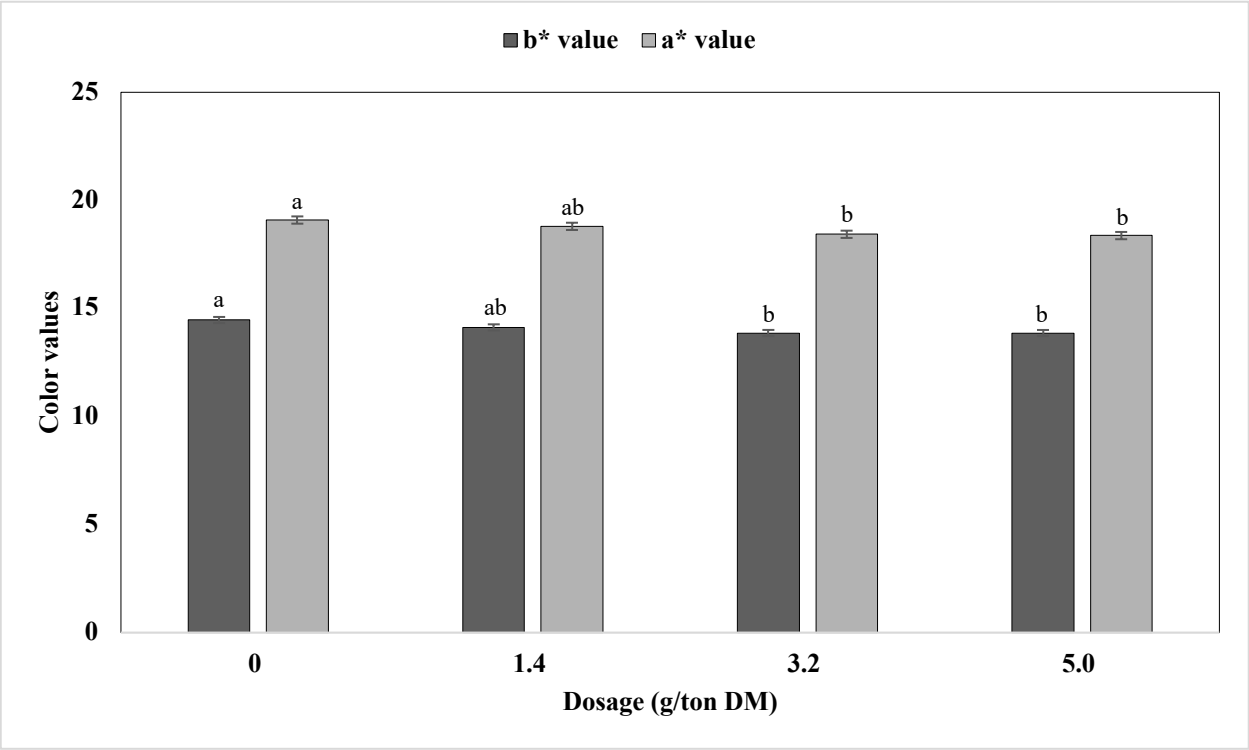

Figure 3.2. Least squares means of a* and b* values of control and Experior (EX) supplemented beef longissimus muscle (n = 534) by the main effect of dose (0, 1.4, 3.2, 5.0 g/ton on DM basis) averaged over 3 feeding durations (FD) (28, 56, 84 d) from a subset of carcasses grading USDA Low Choice.

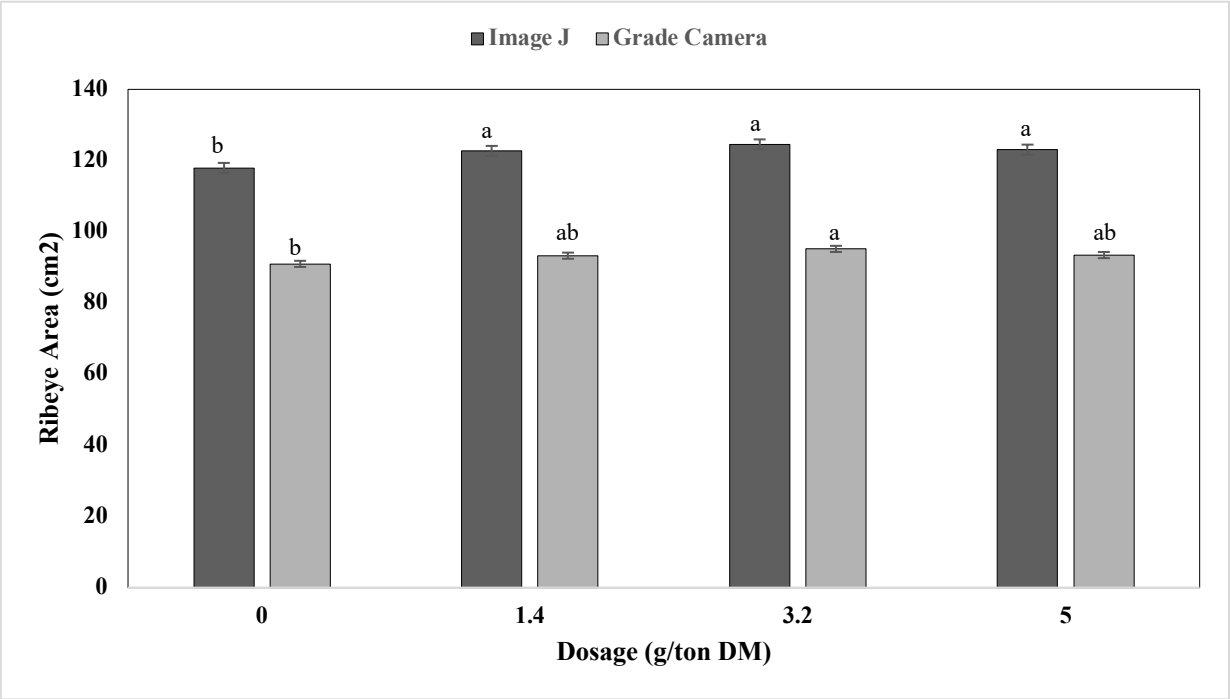

Figure 3.3. Least squares means of control and Experior (EX) supplemented beef longissimus muscle area (LMA) from a USDA grade camera (n = 531) and Image J (n = 536) by the main effect of dose (0, 1.4, 3.2, 5.0 g/ton on DM basis) averaged over 3 feeding durations (28, 56 ,84 d) from a subset of carcasses grading USDA Low Choice.

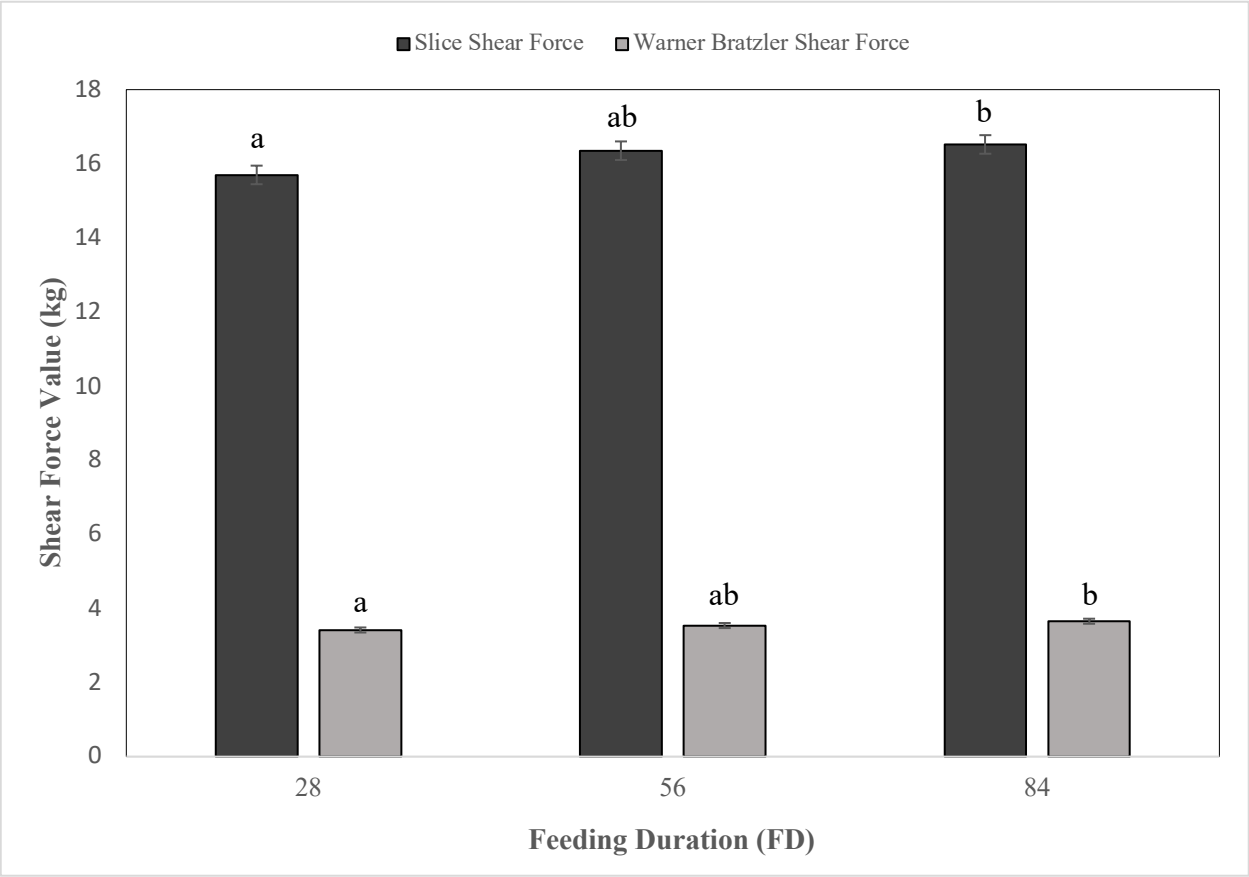

Figure 3.4. Least squares means of Warner-Bratzler shear force (WBSF) and Slice shear force (SSF) values by the main effect of feeding durations (FD) (28, 56, 84 d) averaged over dose (0, 1.4, 3.2, 5.0 g/ton DM basis) and all post mortem aging periods (0, 7, 14, 21, 28 d) (n= 2664).

CHAPTER 4 - MUSCLE PROTEOME CHANGES ASSOCIATED WITH THE SUPPLEMENTATION OF LUBABEGRON ON FINISHED FEEDLOT STEERS

4.1 Introduction

The term proteome is defined as the protein complement of a genome (Wilkins et al., 1996); thus, proteomics refers to characterizing the complements of proteins that are expressed in a cell or tissue type (Bendixen, 2005a). The aim of proteomics is to identify all proteins, their biological activity, post-translational modifications and interactions in a cell and to identify changes in the proteome as a response to altered biological conditions (Zamaratskaia & Li, 2017). An additional objective of proteomics is to discover molecular markers that would allow for a more accurate understanding of particular mechanisms (Bendixen, 2005a). The proteome can be perceived as the molecular relationship between the genome and the functional quality characteristics (phenotype) of meat under certain environmental and processing systems (Hollung et al., 2007).

Meat quality is directly affected by pre- and post- harvest conditions, as well as processing and storage systems.Hence, proteomics is a promising approach in gaining knowledge on the underlying mechanisms that both directly and indirectly affect meat quality (Zamaratskaia and Li, 2017). Bendixen (2005a) and Bendixen (2005b) suggested that with the ability to understand the analytical components and characteristics of the proteome in relation to meat quality and processing systems will ultimately lead to a better understanding and a greater ability to optimize the conversion of muscle to meat.

Beta-adrenergic agonists (βAAs) such as Ractopamine Hydrochloride (RAC) and Zilpaterol hydrochloride (ZH) are utilized as feed additives in food-animal production systems, where they have been proven to increase feed efficiency, live weight gain, carcass yields and

leanness in cattle and swine (Avendaño-Reyes et al., 2006; Elam et al., 2009; Baxa et al., 2010; Scramlin et al., 2010; Arp, 2012). Increased leanness and carcass weights associated with βAA's supplementation are primarily attributed to muscle accretion (Quinn et al., 2008; Bryant et al., 2010; Boler et al., 2012; Edenburn et al., 2016), lipolysis (Mills et al., 2003), and an increase in myofibrillar protein synthesis. It was first established by Vestergaard et al. (1994) that the βAA (Cimaterol) increased the percentage of TypeIIA fibers at the expense of Type I fibers, shifting the metabolism from primarily oxidative to oxidative-glycolytic. Similar shift in muscle fiber type in response to the supplementation of RAC have been reported by several other researchers (Seideman and Crouse, 1986; Gonzalez et al., 2009; Kellermeier et al., 2009; Garmyn et al., 2014; Kim, 2018).

Previous studies demonstrated that βAA supplementation influences the proteome profile of meat. For example, Costa-Lima et al. (2015) examined the influence of RAC on the proteome profile of pork L*ongissimus thoracis* muscle and reported five of the seven differential proteins being over-abundant in RAC (serum albumin; SA, carbonic anhydrase-3; CA3, L-lactate dehydrogenase A chain; LDHA, Fructose-biphosphate aldolase A; ALDOA, myosin light chain 1/3; MLC1/3). Therefore, suggesting that RAC influences the expression of enzymes that are involved in glycolytic metabolism. Further, Wu et al. (2017) examined the effect of RAC on pork semimembranosus muscle and reported that RAC influenced the abundance of proteins involved in oxygen transport, chaperone activity, and plasma membrane repair in sarcoplasmic proteins, which is suggested to be the result of a fiber type shift from Type I oxidative to Type II glycolytic. While these studies have been on pork, Kim (2018) examined the proteome changes in *Longissimus lumborum* muscle of beef supplemented with RAC and reported that dietary RAC influenced the abundance of proteins related to muscle structure development, chaperone activity,

oxygen transport, and glycolysis. These authors concluded that RAC supplementation influenced the expression of enzymes associated with muscle development and muscle fiber type shift.

Ractopamine is a β2AA, which implies that the binding and activation of the β2 receptor without being able to block the β1 or β3 receptors. A novel feed additive known as Lubabegron (Experior; EX) developed by Elanco is considered a β-adrenergic agonist/antagonist (i.e., modulator) which implies its ability to activate the β3 receptor while antagonizing (i.e., blocking) the β1 and β2 receptors. In addition, the β3 receptor (R) is unique compared to the β1 and β2R's, with its assembly being in the fourth intracellular loop rather than the third and are primarily within adipocytes (Fiems, 1987; Strosberg, 1992; Nisoli et al., 1996; Mersmann, 1998; Chikuni et al., 2008). Due to its location, there are fewer opportunities for the inactivation of the β3R binding site by phosphorylation (Arch and Ainsworth, 1983; Strosberg, 1992; Emorine et al., 1994; Langin et al., 1995), thus, reducing the likelihood of desensitization compared to the other two βAAs (Mersmann, 1998).

Previous studies demonstrated that EX can improve carcass characteristics (Chapter 2) of feedlot steers without adversely affecting the meat quality attributes (Chapter 3). However, the effect of EX on muscle proteome profile has not yet been examined. Therefore, the objective of this study was to examine the changes in proteome profile of beef *Longissimus lumborum* muscle in response to EX supplementation. While previous studies (Costa-Lima et al., 2015; Wu et al., 2017; Kim, 2018) utilized two-dimensional gel electrophoresis (2-DE), the current study utilizes a gel free, chemical labelling approach know as tandem mass tag (TMT) coupled with high resolution mass spectrometry to explore the proteome changes.

4.2 Materials and Methods

4.2.1 Animal Production, Carcass Fabrication and Aging

Two thousand one hundred and sixty British and European Continental crossbred steers were housed and fed at the Johnson Research Center in Northwestern Idaho. Experimental design called for a 4 x 3 factorial comprised of 2 factors; dose (0, 1.4, 3.2, 5.0 g/ton) and duration (28, 56, 84 d) in a complete randomized block design. Within each duration group, 15 pens (12 hd/pen) were assigned to each dosage level (0, 1.4, 3.2, 5.0 g/ton), resulting in 60 pens per duration group and 180 total pens across all durations. Three blocks were assigned to each study cycle, where each block consisted of 12 pens (12 hd/pen, 3 pens per treatment group) resulting in a total of 432 steers (36 pens) for each study cycle. Additionally, all steers included in this study were have never allowed to receive an implant or had to be explanted on or before day 99 (14 d prior to the start of the 84 d feeding duration group). All steers were fed a total mixed ration (TMR) including Monensin (40 g/ton) and Tylosin (8 g/ton) by day ~105 and were switched from a basal to a finishing ration at the start of the durational period (day ~85, day ~57, or day ~29) that would contain either 0, 1.4, 3.2 or 5.0 g/ton of LY488756 otherwise known as Lubabegron (Experior®, Elanco Animal Health; EX) (100% dry matter basis).

A sample population (N = 540) of carcasses grading USDA Low Choice (SM00-99) were selected for further evaluation. Three carcasses per pen were chosen based on their USDA quality grade Striploins were fabricated into 5 sections and randomly assigned to five postmortem aging times (1, 7, 14, 21, 28 d). Striploin steaks that were not subjected to postmortem aging (postmortem aging day 1) were utilized for proteome analysis.

4.2.2 Product Description and Methodology

Approximately 200 mg of 25 beef samples were submitted for Tandem Mass Tag (TMT) 6plex labeling, peptide fractionation and protein identification & quantitation via liquid chromatography – mass spectrometry/ mass spectrometry (LC-MS/MS). Cattle pens were assigned to one of 12 treatment groups that were comprised of 4 doses (control (No EX supplementation); 0, 1.4; L , 3.2; M, and 5.0; H g/ton DM basis) and 3 durations of feeding (28, 56, or 84 d). Four out of the twelve treatment groups were chosen for analysis to be able to observe the effects of the treatment extremes. Proteome analysis was be performed on muscle samples obtained from six striploins (n = 6) (or n =7 for C-84) from four of the selected treatment groups (C - 84, H - 84, H - 28, and L - 28) as summarized in Table 4.2.

A MixQC pool was made by combining an equal amount of protein from each sample. This MixQC was then separated into five aliquots for reduction/alkylation, digestion and labeling. It was then pooled and re-aliquoted before multiplexing. All other samples were randomized into 5 TMT sets and assigned a TMT label.

4.2.3 Muscle Proteome Sample Preparation

4.2.3.1 Protein Extraction and Quantification

Approximately 200mg of muscle per sample was provided in 5mL tubes appropriate for use in the Bullet Blender 5 Storm (Next Advance). Lysis Buffer (2.5% SDS, 1X HALT protease inhibitor, 75mM TEAB) was freshly prepared. To each sample, approximately equal volume of 3.2mm stainless steel beads (6 beads) and 500µl lysis buffer were added. Lysis/homogenization were achieved using speed 10 for 3 min followed by speed 12 for 3 min. Two hundred and fifty µl additional lysis buffer was added to each sample followed by transfer to

a 1.5mL microcentrifuge tube. Bullet Blender tubes were rinsed with 500µl lysis buffer which was subsequently transferred to the corresponding sample tube. Samples were then incubated at 100°C for 20 mi. After cooling on ice, samples were centrifuged at 5000xg for 5 min to pellet intact cells and debris. Small aliquots were diluted 1:50 and measured for total protein content using the Pierce BCA Protein Assay Kit (ThermoFisher Scientific) using manufacturer instructions.

4.2.3.2 Protein Reduction-Alkylation and Digestion

Preparation of all solutions and procedures were as described in the TMT 6-plex kit instructions (ThermoScientific). Briefly, 100µg protein from each sample (or MixQC) was aliquoted and raised to 100µl total volume using 100mM triethyl ammonium bicarbonate (TEAB). For reduction of disulfide bonds, Tris(2-carboxyethyl)phospine (TCEP) was added to 9.5mM final concentration and incubated 55°C for an h. Free cysteines were then alkylated at room temperature using 17.9mM final concentration iodoacetamide (IAM) for 30 min in the dark. Six volumes of ice cold acetone were then added and protein precipitation occurred overnight at -80°C. Precipitate was harvested at 8000xg, 4°C, for 10 min. Supernatants were decanted into fresh tubes and pellets were allowed to air dry under a sheet of foil. Pellets were then reconstituted in 100ul TEAB and digested with 2.5µg Trypsin at 37°C overnight.

4.2.3.3 Peptide Labeling and Cleanup

After digestion, absorbance at 205nm was measured on a NanoDrop (ThermoScientific) and total peptide concentration was subsequently calculated using an extinction coefficient of 31 (Scopes, 1974). Eighty-one µg peptide from each sample was aliquoted and raised to 100µl using TEAB. All TMT label reagents were allowed to equilibrate to room temperature followed by the addition of 41µl liquid chromatography – mass spectrometry (LC-MS) acetonitrile and occasional vortexing over 5 min. All vials of like labels were then pooled together and mixed via vortex. Forty

one µl of TMT label was added to each sample and incubated at room temperature for 1 h. Quenching was achieved by addition of hydroxylamine (0.37% final) and 15 min additional incubation. Following quenching, samples were pooled into 5 TMT. Pooled, labeled peptides were cleaned up using TT1000C18 TopTips (PolyLC) using manufacturer's instructions and centrifugation at 3000 rpm. Solvents were: Activation: 50% Acetonitrile (ACN); Equilibration and Wash: 5% ACN, 0.5% trifluroacetic acid (TFA) and Elution: 70% ACN, 0.1% formic acid (FA). Eluates were dried using a Savant speedvac and reconstituted in 300µl 0.1% TFA.

4.2.3.4 Peptide Fraction

After cleanup and reconstitution, peptide concentration was measured on the NanoDrop as described above. Fifty µg total peptide was subjected to high pH fractionation using spin columns from the Pierce High pH Reversed-Phase Peptide Fractionation Kit following manufacturer's instructions for TMT labeled peptides. The wash solution consisted of 0.1% Triethylamine (TEA) as diluent and ACN added to 5% final concentration. Elution solutions contained 0.1% TEA as diluent with ACN added to 10-50% final concentration. Stepwise fractionation took place using 300µl elution solution and spinning at 3000xg for 2 min at room temperature. A total of 8 fractions were collected and subsequently dried in a Savant speedvac, reconstituted in 12µl of 5% ACN, 0.1% FA, and quantified as described above using the NanoDrop. Fractions 1 and 2 were markedly lower in concentration and were pooled to facilitate injection of equal total peptide per run.

4.2.4 Mass Spectrometry Analysis

The 7 fractions from each TMT set were block randomized and injected in a randomized set order. A total of 0.7µg of peptides were purified and concentrated using an on-line enrichment column (Waters Symmetry Trap C18 100Å, 5µm, 180 µm ID x 20mm column). Subsequent

chromatographic separation was performed at a flow rate of 350 nanoliters/min on a reverse phase nanospray column (Waters, Peptide BEH C18; 1.7µm, 75 µm ID x 150mm column, 45°C) using an 85 min linear gradient from 5%-40% buffer B (100% ACN, 0.1% formic acid) followed by 40-85% buffer B over 7 min. Peptides were eluted directly into the mass spectrometer (Orbitrap Velos Pro, Thermo Scientific) equipped with a Nanospray Flex ion source (Thermo Scientific) and spectra were collected over a *m*/*z* range of 400–1500, positive mode ionization. The top 10 ions with charge state +2 or higher were accepted for MS/MS using a dynamic exclusion limit of 1 MS/MS spectra of a given *m*/*z* value for 30s (exclusion duration of 120s). The instrument was operated in FT profile mode detection for both MS and MS/MS detection (resolution of 30,000 and 7,500 respectively). Fragmentation was via HCD with a normalized collision energy set to 35%. Compound lists of the resulting spectra were generated using Xcalibur 3.0 software (Thermo Scientific) with a S/N threshold of 1.5 and 1 scan/group.

4.2.5 Statistical Analysis

Tandem mass spectra were extracted, charge state deconvoluted and deisotoped by ProteoWizard MsConvert (version 3.0). Spectra from all samples were searched using Mascot (Matrix Science, London, UK; version 2.6.0) against the Uniprot_Bovine_rev_102819 database (75764 entries), assuming the digestion enzyme trypsin. Mascot was searched with a fragment ion mass tolerance of 0.020 Da and a parent ion tolerance of 20 PPM. Carbamidomethyl of cysteine was specified in Mascot as a fixed modification. Deamidation of asparagine and glutamine, oxidation of methionine and TMT6plex of lysine and the n-terminus were specified in Mascot as variable modifications.

Search results from each TMT set were subjected to MuDPIT, imported and combined using the probabilistic protein identification algorithms developed by Keller et al (2002) which

was implemented in the Scaffold software (version Scaffold_4.10.0, Proteome Software Inc., Portland, OR) (Searle et al., 2008). Peptide thresholds were set (85%) such that a peptide FDR of 0.07% was achieved based on hits to the reverse database (Käll et al., 2008). Protein identifications were accepted if they could be established at greater than 99.9% probability (0.9%FDR) and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (Nesvizhskii et al., 2003). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

Channels were corrected using values supplied by ThermoScientific (Lot UG285312) according to the algorithm described in Shadforth et al. (2005). Normalization was performed iteratively (across samples and spectra) on intensities, as described in (Oberg et al., 2008). Medians were used for averaging. Spectra data were log-transformed, pruned of those matched to multiple proteins and those missing a referenced value, and weighted by an adaptive intensity weighting algorithm. Of 72807 spectra in the experiment at the given thresholds, 54121 (74%) were included in quantitation. Proteins that exhibited at least 1.5 fold variation between the treatment groups and had a statistical difference was considered as differentially abundant.

Mann-Whitney and Kruskal-Wallis analyses were performed within the Q+S module of Scaffold on Log2 normalized intensities. Benjamini-Hochberg computation of the FDR and subsequent correction of p-values were performed as explained in (Benjamini and Hochberg, 1995).

4.3 Results and Discussion

4.3.1 Muscle Proteome Analysis

Six muscle samples from striploins were randomly selected from 4 treatment groups which consisted of C84, L28, H28, and H84 (N = 25). A total of 10 differentially abundant proteins ($P < 0.05$) were identified from six treatment comparisons (H84 vs. C84, H28 vs. C84, L28 vs. C84, H84 vs. H28, H84 vs. L28, H28 vs. L28) and are represented in Table 4.1, along with their protein name, accession number, abbreviation (string code) and differential abundance ratio. The identified proteins were involved in muscle contraction/calcium signaling (myosin light chain 3 and 6B, ankyrin repeat domain 2, cysteine and glycine-rich protein 3, and gelsolin), transport (hemoglobin subunit alpha and beta and alpha-1-acid glycoprotein), growth factor (mimecan), and proteasomes (proteasome activator complex subunit 1).

4.3.2 Proteins Related to Muscle Contraction or Calcium Signaling

Four differential proteins (myosin light chain 3 and 6B, ankyrin repeat domain 2, gelsolin) were involved in muscle contraction while one (cysteine and glycine-rich protein 3) was associated with calcium signaling (Table 4.1). Among these proteins, myosin light chain 3 (MYL3) and 6B (MYL6B) are both structural proteins (Yu et al., 2017) and are closely associated with meat quality. These proteins have been reported to be a predictor of post mortem proteolysis (Malheiros et al., 2019). Zhang et al. (2010) demonstrated that the deficiency in MYL3 was associated with greater intramuscular fat (IMF) deposition in the longissimus dorsi (LD) of cattle. Similarly, Poleti et al. (2018) reported a lower abundance of MYL6B in cattle LD muscle that was highly marbled. Comparable to previous studies, in the current study, MYL6B was more abundant ($P < 0.05$) in

L28 when compared to C84 and H28. These results suggest that an increase in IMF may be associated with an under expression of MYL6B, and not with MYL3.

Previously, Yu et al. (2017, 2018) reported an over-abundance of MYL3 and a lower expression of MYL6B in beef *psoas major* (PM) as compared to the *longissimus lumborum* (LL) in the early postmortem stages. Typically, PM is considered to have greater tenderness compared to LL (Nair et al., 2019). The overabundance of MYL3 in L28 compared to H84 corresponded with aa decrease ($P < 0.05$) in Warner-Bratzler shear force (WBSF). Furthermore, according to Malheiros et al. (2019), the oxidative damage in tender meat was greater than tough meat due to an over-abundance of MYL3 and MYL6B. In relation to tenderness values, there were no statistical differences in WBSF for any treatment comparisons made for MYL6B (L28 vs.C84 and H28 vs. L28; Chapter 2). Both Costa-Lima et al. (2015) and Wu et al. (2017) reported an over-abundance of MYL1/3 in the *longissimus thoracic* and *semimembranosus muscles* produced from RAC supplemented hogs. Furthermore, Wu et al (2017) suggested that the over expression of MLC1/3 in RAC muscle may be due to the increased protein synthesis and has been reported to have a positive correlation to shear force values of pork *longissimus* muscle (Hwang et al., 2005).

Ankyrin repeat domain 2 (ANKRD2), regardless of duration, was over expressed in the lowest dose (L28) when compared to the highest dose (H28). Expression of ANKRD2 was greater in swine breeds with exponential growth rates (Sun et al., 2011) and an increased expression of ANKRD2 also was found in fast-twitch (Type II) skeletal muscle fibers as opposed to slow-twitch (Type 1) muscle fibers (Nakamura et al., 2002). The ANKRD2 can directly interact with p53 and enhance its activity (Kojic et al., 2004), and increased ANKRD2 expression can induce muscle cell apoptosis by increased caspase 3 activity (Bean et al., 2008). Furthermore, Cenni et al. (2019) reported that the over-expression of ANKRD2 leads to apoptosis and is

mediated by p53. The results of the current study indicate that when Experior is supplemented at a lower dose, it could increase the rate of cell death and increase the rate of postmortem proteolysis, thereby decreasing postmortem aging time.

Cysteine and glycine-rich protein 3 (CSRP3), also referred to as muscle LIM protein (MLP), is an important scaffold protein in the sarcoplasm that is related to calcium homeostasis (Esposito et al., 2000; Su et al., 2001; Gupta et al., 2008; Kemecsei et al., 2010). The CSRP3 was over abundant ($P < 0.05$) in H84 when compared to both C84 and H28 (Table 4.1). The protein CSRP3 is known to play a crucial role in muscle development and maintenance; therefore, suggesting that it also regulates autophagy which is a protective mechanism against apoptosis or cell death (Cui et al., 2020). Additionally, CSRP3 is associated with metabolic disorder, cell death, or factors related to cell death (van den Bosch et al., 2005; Rashid et al., 2015). As described, CSRP3 was under expressed in cattle that were supplemented with EX at either a low/control dose and/or short feeding duration compared to the highest dose for longest feeding duration. Previous research has indicated that the abundance of CSRP3 was positively associated with Warner-Bratzler shear force (WBSF) of beef aged for 14 d (Zapata et al., 2009). Our previous study (Chapter 3) indicated that regardless of postmortem aging times, control steaks were more tender than steaks from cattle supplemented with EX at high dose (5.0 g/ton) for 84 days (H84). The over-expression of CSRP3 in H84 did not correlate to tenderness differences (Chapter 3).

In the present study, Gelsolin (GSN) was detected as being in higher abundance ($P < 0.05$) in H84 when compared to C84. GSN is a calcium-regulated, actin-modulating protein that binds to the ends of actin monomers or filaments. Consequently, the association between GSN and its relation to meat quality has not been determined. Nonetheless, it has been reported that GSN can prevent apoptosis by inhibiting apoptotic mitochondrial change (Azuma et al., 2000; Koya et al.,

2000; Kusano et al., 2000), decrease activation of caspase-3 (Ohtsu et al., 1997; Harms et al., 2004). On the other hand, cleaved or fragmented gelsolin can contribute to apoptosis (Kothakota et al., 1997; Geng et al., 1998; Azuma et al., 2000). Although our previous research (Chapter 3) has reported that C84 was consistently more tender at all postmortem aging periods than H84, the potential role of GSN in that is not clearly understood.

4.3.3 Transport Proteins

Among the transport proteins identified, hemoglobin subunit alpha (HBA) and hemoglobin subunit beta (HBB) were under-expressed ($P < 0.05$) in H28 compared to L28 and H84 (Table 4.1). The primary function of hemoglobin is to transport oxygen (Hsia, 1998; Wu et al., 2017). Therefore, within postmortem muscle, the contribution of hemoglobin to meat quality attributes is negligible. Moreover, hemoglobin is left in trace amounts in the muscles after exsanguination (Warriss and Rhodes, 1977). However, the difference in the detected levels of hemoglobin could be reflective of their muscle fiber types. Yu et al. (2017) also reported an over-abundance of HBA and HBB in beef PM in contrast to LL, with LL being a glycolytic muscle and PM being an extreme oxidative muscle (Kirchofer et al., 2002). Similarly, in the current study, samples evaluated consisted primarily of Type II glycolytic muscle fibers (Chapter 2). In contrast, Wu et al. (2017) reported that muscles from RAC fed cattle resulted in HBB being under-expressed compared to those obtained from a negative control. However, the researchers did not examine muscle fiber type response with RAC supplementation. Previous research has indicated that the muscle fiber type shifted from Type I to Type IIA using RAC supplementation in steers (Gonzalez et al., 2009) and hogs (Paulk et al., 2014). Contrary to Wu et al. (2017), both HBA and HBB were observed in the current study as a response to EX supplementation. Interestingly, the high dose (5.0g/ton) for 28 days resulted in lower HBA and HBB expression than the low dose (1.4g/ton) for 28 days or

the high dose (5.0 g/ton) for 84 days. It has been reported that, when isoprenaline (nonselective beta-agonist) was supplemented, ventricular weight increased by 28% in rodent muscle when compared to the controls (Sillau and Philippi, 1987) and it was further suggested by Wright et al. (1981) that revascularization is a result of increased blood flow to skeletal muscle. Moreover, Taylor and Tang (1984) reported an approximate 20% increase in ventricular DNA. The over-abundance of HBA and HBB that was observed in RAC LM muscle could possibly be attributed to increased vascularization as a response to βAA administration. The increased vascularization could lead to a greater amount of hemoglobin being captured with the capillaries of postmortem muscle, thereby leading to an adjusted proteome that we observed.

Alpha-1-acid glycoprotein (ORM1) is a transport protein in the blood stream and is known for its ability to bind to various lipophilic and acidic drugs, and it is a major drug carrier protein in plasma (Kremer et al., 1988). In the present study, OMR1 was under expressed ($P < 0.05$) in H28 when compared to C84, L28 as well as H84. The association between ORM1 and meat quality has not been greatly researched. Van Molle et al. (1997) reported that ORM1 plays an important role in the regulation of excess apoptosis or cell death. Beta-3 receptors are primarily located in adipose tissue and are involved in the regulation of lipolysis and thermogenesis (Sawa and Harada, 2006). Furthermore, the capacity of ORM1 to work as a carrier protein and the ability to transport lipophilic drugs, such as EX, could explain the overabundance of ORM1 within treatments groups that were associated with the high dose. Therefore, EX supplementation may increase the likelihood of the expression of ORM1.

4.3.4 Growth Factor

Mimecan (OGN), also referred as osteoglycin, is an extracellular matrix proteoglycan that is present in the bone matrix, cartilage cells, as well as connective tissues, and is critical for cellular

growth, differentiation and migration (Hu et al., 2005). Mimecan was first identified as a small leucine-rich proteoglycan (SLRP) in bovine bone, and is known for the ability to bind to a multitude of growth factors, cell receptors, insulin growth factor receptors, as well as other matrix proteins (Deckx et al., 2016). It was detected at a higher abundance ($P < 0.05$) in L28 than C84. Experior is a βAA that can increase protein accretion and growth performance in cattle. The overabundance of OGN seen in in the muscle produced by EX supplemented cattle produced muscle compared to control muscle could be related to the increased growth performance observed in the EX supplemented cattle. This differential abundance of Mimecan was only observed in L28 (low dose for short duration), but not in samples obtained from cattle provided a higher dose for same (H28) or longer (H84) duration. te Pas et al. (2013) reported that the abundance of OGN is positively associated with drip loss in pork carcasses and suggested that it could be a potential biomarker for drip loss prediction (te Pas et al., 2013). However, in the present study, drip loss was not evaluated. More importantly, cooking loss was not influenced by the EX supplementation.

4.3.5 Proteasome Activation

Proteasome activator complex subunit 1 (PSME1) also is referred to as the alpha subunit of the PA28 complex (PA28α) and is a large protein complex that is responsible for energy-dependent degradation of peptides (Tanaka, 2009; Cascio, 2014). The proteasome is made up of two subcomplexes: a catalytic core known as the 20S proteasome and a 19S regulatory particle (RP) terminal. The 19S RP forms conical caps at the ends of the 20S proteasome, creating the 26S proteasome (Coux et al., 1996) that is responsible for ATP-dependent degradation of proteins (Rechsteiner et al., 2000). The 26S proteasome degrades unneeded or damaged proteins that are marked by ubiquitin.

In the current study, PSME1 was over-abundant ($P < 0.05$) in H84 compared to C84. The carcass yield characteristics of H84 were greater than C84 (chapter 2) suggesting that the greater PSME observed in H84 could be in response to greater protein synthesis and repair happening in H84 during the growth. Moreover, a negative correlation between PSME1 abundance in beef LL muscle and intramuscular fat content was reported by Bazile et al. (2019). Our research indicated that cattle fed high a dose (5.0 g/ton) of EX for 84 days resulted in decreased intramuscular fat as evidenced by a decrease in the percentages of carcasses grading USDA Prime and Premium Choice when compared to carcasses of negative control cattle (C84).

4.4 Conclusion

Results of this study indicated that the proteome profile varied with EX dosage and feeding duration. The EX supplementation influenced expression of proteins involved in muscle contraction, calcium signaling, transport, growth factor, and proteasome activation. Among these, MYL3 was associated with an improved tenderness and carcass grading, which could be reflective of increased intramuscular fat content. The proteins identified (HBA, HBB, and ORM1) were suggestive of increased vascularization in muscles as a response to EX supplementation. Further research is necessary to understand the exact cellular mechanistic pathways in response to EX supplementation.

Table 4.1. Differentially abundant proteins between beef longissimus lumborum muscle from different treatments from a subset of carcasses grading USDA Low Choice ($P < 0.05$).

Protein Description	Accession Number	Abbreviation (string code)	Fold Change (L28:C84)	Fold Change (H28:C84)	Fold Change (H84:C84)	Fold Change (H28:L28)	Fold Change (H84:L28)	Fold Change (H84:H28)
Muscle contraction/calcium signaling								
Myosin light chain 3	MYL3_BOVIN	MYL3					0.66	
Myosin light chain 6B	Q148H2_BOVIN	MYL6B	1.54			0.57		
Ankyrin repeat domain 2	F1MX12_BOVIN	ANKRD2				0.65		
Cysteine and glycine-rich protein 3	CSRP3_BOVIN	CSRP3			1.61			1.64
Gelsolin	GELS_BOVIN	GSN			1.56			
Transport protein								
Hemoglobin subunit alpha	HBA_BOVIN	HBA				0.64		1.51
Hemoglobin subunit beta	HBB_BOVIN	HBB				0.57		1.66
Alpha-1-acid glycoprotein	A1AG_BOVIN	ORM1		0.54		0.54		2.43
Growth factor								
Mimecan	MIME_BOVIN	OGN	1.56					
Proteasome								
Proteasome activator complex subunit 1	PSME1_BOVIN	PSME1			1.58			

Six different steaks were randomly selected 4 different treatment groups that consisted of the control dose at feeding duration 84 (C84), the low dose (1.4 g/ton) at feeding duration 28 (L28), and two treatments within the high dose (5.0 g/ton) at feeding durations 28 and 84 d (H28 and H84). A total of 10 differentially abundant proteins ($P < 0.05$) were identified from the six following comparisons (H84 vs. C84, H28 vs. C84, L28 vs. C84, H84 vs. H28, H84 vs. L28, H28 vs. L28).

Table 4.2. Randomized distribution of samples amongst 5 TMT sets. Set number and TMT label used are indicated

PMF ID	Sample Name	TMT Group	TMT Label
MixQC1	MixQC1	I	126
929	1974_C_84	I	127
328	1224_C_84	I	128
583	1966_H_84	I	129
994	1310_H_28	I	130
923	789_H_28	I	131
MixQC2	MixQC2	II	126
605	1303_H_28	II	127
330	2212_C_84	II	128
797	1990_L_28	II	129
570	1126_L_28	II	130
602	887_C_84	II	131
MixQC3	MixQC3	III	126
358	837_H_28	III	127
336	560_L_28	III	128
378	2429_H_84	III	129
601	1942_C_84	III	130
755	2046_C_84	III	131
MixQC4	MixQC4	IV	126
725	1682_H_28	IV	127
772	1911_H_84	IV	128
184	1347_H_84	IV	129
344	1120_L_28	IV	130
607	507_H_84	IV	131
MixQC5	MixQC5	V	126
163	200_H_28	V	127
986	1270_L_28	V	128
110	280_C_84	V	129
190	1451_H_84	V	130
107	60_L_28	V	131

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Appendix A - Data Collection Sheets

Appendix A 1. Low Choice Strip Loin Collection

Date: Low Choice Strip Loin Selection ELA 1800074 Page 1 of___

Kill Lot	Sequence	Pen Assignment	Low Choice √
19	90265	92	
19	90267	94	
19	90269	94	
19	90271	93	
19	90273	93	
19	90275	93	
19	90277	94	
19	90279	93	
19	90281	93	
19	90283	93	
19	90285	93	
19	90287	94	
19	90289	94	
19	90291	92	
19	90293	94	
19	90295	92	
19	90297	94	
19	90299	92	
19	90301	92	
19	90303	92	
19	90305	94	
19	90307	93	
19	90309	94	
19	90311	94	
19	90313	93	
19	90315	94	
19	90317	93	
19	90319	92	
19	90321	93	
19	90325	92	
19	90327	92	
19	90329	94	
19	90331	92	
19	90333	92	
19	90335	92	
19	90337	93	

92

93

94

Recorded By:_____ Observed By:_____

Appendix A 2. Colorimeter Data Sheet

Date_____ **ELA1800074** Page 1 of ____

Colorimeter Data Sheet **Pasco, WA**

#	Sequence #	Strip Loin #	L*	a*	b*	L* SD	a* SD	b* SD	Comment
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									

Observed by:_____ Recorded by:_____

Appendix A 3. Kidney Pelvic and Heart Fat and Preliminary Yield Grade Data

Date_____ **ELA1800074** Page 1 of _____

Carcass Data Sheet **Pasco, WA**

#	Lead Side Cxs ID	HCW	Adjusted PYG	KPH Fat	Comment
1	90265				
2	90267				
3	90269				
4	90271				
5	90273				
6	90275				
7	90277				
8	90279				
9	90281				
10	90283				
11	90285				
12	90287				
13	90289				
14	90291				
15	90293				
16	90295				
17	90297				
18	90299				
19	90301				
20	90303				
21	90305				
22	90307				
23	90309				
24	90311				
25	90313				
26	90315				
27	90317				
28	90319				
29	90321				
30	90325				
31	90327				

Observed by:_____ Recorded by:_____

Appendix A 4. Carcass Maturity Carcass Data Sheet

Date_____ **ELA1800074** Page 1 of _____

Carcass Data Sheet **Pasco, WA**

#	Lead Side Cxs ID	HCW	Skel. Maturity	Lean Maturity	Comment
1	90265				
2	90267				
3	90269				
4	90271				
5	90273				
6	90275				
7	90277				
8	90279				
9	90281				
10	90283				
11	90285				
12	90287				
13	90289				
14	90291				
15	90293				
16	90295				
17	90297				
18	90299				
19	90301				
20	90303				
21	90305				
22	90307				
23	90309				
24	90311				
25	90313				
26	90315				
27	90317				
28	90319				
29	90321				
30	90325				
31	90327				

Observed by:_____Recorded by:_____

Appendix A 5. Ribeye Area and Quality Grade Carcass Data Sheet

Date_____ **ELA1800074** Page 1 of _____

Carcass Data Sheet **Pasco, WA**

#	Lead Side Cxs ID	HCW	REA (higher of 2 sides)	Marbling Score (higher of 2 sides)	Comment
1	90265				
2	90267				
3	90269				
4	90271				
5	90273				
6	90275				
7	90277				
8	90279				
9	90281				
10	90283				
11	90285				
12	90287				
13	90289				
14	90291				
15	90293				
16	90295				
17	90297				
18	90299				
19	90301				
20	90303				
21	90305				
22	90307				
23	90309				
24	90311				
25	90313				
26	90315				
27	90317				
28	90319				
29	90321				
30	90325				
31	90327				

Observed by:_____ Recorded by:_____

Appendix B - Sensory Ballot

Appendix B 1. Qualtrics Trained Sensory Ballot

Enter Name:

Sample 1:

Initial Tenderness:

Sustained Tenderness

Initial Juiciness

Sustained Juiciness

Beef Flavor ID

Fat-like

Brown/ Roasted

Bloody/ Serumy

Metallic

Oxidized

Liver

Sour

Bitter

Umami