AN EXAMINATION OF THE EFFECTS OF DIETARY RUMEN-PROTECTED HISTIDINE SUPPLEMENTATION ON FINISHING BEEF CATTLE GROWTH, CARCASS, AND MEAT QUALITY PARAMETERS

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Authorization to Submit Thesis

This thesis of Breann N. Sandberg, submitted for the degree of Master of Science with a major in Animal Science and titled "An Examination of the Effects of Dietary Rumen-Protected Histidine Supplementation on Finishing Beef Cattle Growth, Carcass, and Meat Quality Parameters," has been reviewed in final form. Permission, as indicated by the signatures and dates given below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

This study examined the effects of dietary rumen-protected histidine (HIS) supplementation in beef cattle on growth and carcass traits/product quality. Three levels of daily HIS were tested over a 60-d finishing period in 48 cross-bred steers. Cattle were randomly allocated into eight pens (six hd/each) and fed using Calan gates. Morning feed was top-dressed with the HIS; control (no HIS), low HIS (50g/hd/d), or high HIS (100g/hd/d). Individual intakes were recorded, and feed and orts were analyzed. Post-harvest, one *longissimus lumborum* and one *gluteus medius* was acquired from each animal and cut into steaks. Steaks were used for retail display to evaluate color, lipid oxidation, cookery data, WBSF, consumer perception and for free histidine, anserine, and carnosine content analysis. HIS supplementation tended improved instrumental and visual color, sensory components, and free HIS content of the lean tissue. In conclusion, RP-histidine treatment may optimize product quality and marketability in beef cattle.

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Dedication

I would like to dedicate this thesis to everyone who has helped me grow and learn through the years, namely my parents and grandparents. Your support and encouragement have been irreplaceable.

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List of Abbreviations

AA	Amino Acid
ADG	Average Daily Gain
AMSA	American Meat Science Association
ANS	Anserine
BF	Back Fat
CARN	Carnosine
CIE	Commission International de l'Eclairage
DMI	Dry Matter Intake
FCR	Feed Conversion Ratio
F:G or G:F	Feed:Gain or Gain:Feed
GM	*Gluteus medius*
HCW	Hot Carcass Weight
hd	Head
HIS	Histidine
HPLC	High Performance Liquid Chromatography
IGF-1	Insulin-like growth factor 1
IMPS	Institutional Meat Purchase Specifications
KPH	Kidney Pelvic and Heart Fat %
LD	*Longissimus dorsi*
LL	*Longissimus lumborum*
LSM	Least Squares Means
LT	*Longissimus thoracis*

LW	Live Weight
MADS	MCM1, agamous, deficiens, and serum response factor
MDA	Malondialdehyde
Mef-2	Myocyte specific enhancer factor
MGA	Melengestrol acetate
MMP	Matrix Metalloproteinase
MP	Microbial protein
MRF	Myogenic regulatory factor
MyoD	Myogenic determination factor
Myf-4	Myogenin
Myf-5	Myogenic response factor 5
NCBA	National Cattlemen's Beef Association
PITC	Phenylisothiocyanate
QG	Quality Grade
RDP	Rumen degradable protein
REA	Rib Eye Area
RP-HIS	Rumen-Protected Histidine
RUP	Rumen undegradable protein
SAS	Statistical Analysis Software
SEM	Standard Error of the Mean
SSF	Slice Shear Force
ST	Somatotropin
TBA	Trenbolone acetate

TBARS	Thiobarbituric Acid Reactive Substances
TEA	Triethanolamine
TMR	Total Mixed Ration
UCP	Uncoupling protein
USDA	United States Department of Agriculture
WBSF	Warner-Bratzler shear force
WHC	Water Holding Capacity
YG	Yield Grade

CHAPTER 1

Review of Literature

Introduction

The focus of my graduate research in animal science is the physiological growth of skeletal muscle in beef cattle, as well as the effects of pre-harvest management on beef product quality and consumer acceptability. Specifically, my studies pertained to the supplementation of beef cattle during finishing with a rumen protected histidine, an amino acid that may at times be limiting and has special pertinence to muscle and meat quality. The forthcoming literature review will outline the current understanding of finishing beef cattle production, meat quality and the relationship that dietary histidine has with respect to lean growth and carcass and meat quality. Additionally, this brief literature review will provide support for our hypotheses and outline the relevance of our observations to the scientific and cattle communities.

In the March 2014 issue of "Beef Magazine", the USDA reported that per-capita annual beef consumption has fallen almost 23 lbs. since 1985. It is expected to fall an additional 4.5 lbs by the year 2023. While the domestic market will still remain an important component of economic sustainability, it is becoming imperative that we expand "beef" export to regions of the world where beef consumption is increasing. This expansion is an essential component of sustainability of the US beef production industry. Increases in feed costs and competition for available high quality protein sources may be causing producers to unknowingly limit their growing livestock of essential dietary amino acids (AA), thus, preventing the animal from reaching true growth potential. Consumer acceptability of the product in terms of flavor profile, color, tenderness, juiciness, and the presence of off-flavors directly impacts the consumption and sale of beef products in the U.S., as well as on export markets.

Since shipping and day from harvest to marketing may rise over the next decade, one product quality parameter; color stability, may become a quality of increasing importance. Optimal color profiles may help to ensure that US beef has the most appealing marketable appearance at new and developing export markets, relative to other competing beef export nations, such as Brazil, Canada, Argentina and Australia. Inevitably, this concept has the potential to improve beef quality and consumer appeal through a cost-effective nutritional pre-harvest intervention. Pre-harvest strategies, such as this, are designed to enhance livestock performance via nutrient alteration, and have the ability to favorably alter the quality aspects of beef. Consequently, this route of product enhancement is perceived as natural and is the most widely accepted producer intervention by consumers (Grunert, 2011).

Skeletal muscle accounts for 40-60% of lean body mass in beef animals and plays important roles in metabolism, systemic physiology, homeorrhesis, and locomotion. In addition to the attainment of physical motion, skeletal muscle facilitates glucose storage (in the form of glycogen), thermoregulation, water regulation, and plays crucial roles in energy metabolism. Ante-mortem lean muscle accretion and growth also provides the template for marketable beef product consumed across the globe as a high-quality source of protein and other nutrients. Thus, the quantity and quality of meat is completely dependent upon that animal's ability to accrete striated muscle mass efficiently in a relatively short time-period. Understanding the concepts of skeletal muscle physiology is important for both the growth of lean muscle, as well as the post-mortem conversion of muscle to meat and the associated changes in metabolism that impact product quality and product marketability.

Pacific Northwest United States Beef Production Scenarios

Currently in the U.S., the beef production system is comprised of several phases: the first, is the cow/calf phase; second is the backgrounding phase; and third is the finishing phase.

The cow/calf phase is usually range/pasture based where the maternal nutrition is forage based and typically lower-quality in nature. Summers are usually spent grazing native grasses and legumes, and winters are typically supplemented with baled forages and a molasses based energy and protein supplement. At this time, the calf spends the first five or six months of life on the dam's milk and slowly graduates to elevated levels of forage inclusion. Some producers may creep feed or supplement the calves minimally during this time. At weaning (usually 5-7 months of age), calves are either fed a forage based diet, or are backgrounded on a low content concentrate diet until the time they are transitioned into a feedlot type system (at about 10-12 months of age).

The finishing phase consists of rapid growth consisting of the transition from muscle growth to increased adiposity. During this time, the calves are fed a graduated high concentrate diet (>60%) and typically implanted with a combination (trenbolone acetate/estradiol) steroid growth promotant. Many feedlots utilize orally administered beta-adrenergic agonists such as Ractopamine hydrochloride (Optaflexx™) for the final 20-28 days of finishing to improve feed conversion efficiency and increase muscle growth. Cattle are usually harvested at 14-18 months of age at about 650 kg body weight (USDA, 2017).

Skeletal muscle growth and development

Muscle growth is defined as an increase in tissue mass (Owens et al., 1993) and is highly influenced by pre-natal development and environment (Du et al., 2010). Prior to birth, hyperplasia is still occurring at high rates allowing for an increase in available muscle fiber

number. Myogenic differentiation involves the sequential expression of transcription factors, such as MyoD, myogenin and Myf-5 that initiate the expression of myosin heavy chain specific genes (Buckingham et al., 2003; Collins et al., 2009). Muscle growth can be divided into two phases: primary myogenesis and secondary myogenesis. Primary muscle fibers form within the first two months of gestation in bovine fetuses, while secondary myofibers, which account for the majority of muscle mass in adults (Beermann et al., 1978), from 2 and 7 months of gestation (Russell & Oteruelo, 1981). There is minimal increase in muscle fiber number post-natally and at this point, muscle growth is mainly due to hypertrophy and the associated deposition of more myofibrillar proteins (Karunaratne et al., 2005; Stickland, 1978). Satellite cells (from fetal myoblasts) surround mature muscle fibers which can then differentiate and fuse with existing fibers to allow for growth and repair (Kuang et al., 2007). As the animals ability to form new fibers post-natally and the mature size of the muscle fiber are limited, a higher number of fibers formed during gestation allows for greater growth potential. If the number of muscle fibers becomes depleted due to a challenge such as nutrient restriction, then the animal may not completely compensate for the loss in growth potential. Additionally, nutrient restriction or fluctuation during late gestation in the dam can reduce muscle size in the progeny (Du et al., 2010b; Greenwood et al., 1999). Post-natally, muscle growth is due in large to hypertrophy of the individual muscle fibers by protein accretion, DNA accretion, and extracellular matrix generation (Figure 1.1) (Allen et al., 1979; Mozdziak et al., 1997). Protein accretion must be carried out via a positive fractional accretion rate: the fractional synthesis rate of protein surpassing the fractional breakdown rate of the existing protein (Figure 1.2) (Kumar et al., 1999; Goll et al., 2008). This increase in accretion must be supported by an increase in myonuclear number and DNA content within that muscle in which a particular myonucleus controls

transcription within its own domain (Adams et al., 2002), although in a state of muscle atrophy, the number of nuclei remain unchanged. Therefore, the number of myonucleii per myofiber dictates the maximum potential for hypertrophic growth, the actual or realized growth is dependent upon all intrinsic and extrinsic environmental factors including nutrient balance, energy demands and health.

The functionality and quality of skeletal muscle is maintained via the constant turnover of protein resulting from synthesis and degradation. These processes are balanced and controlled by many pathways that target particularly the rapamycin (mTOR) pathway and the ubiquitin-proteasome pathway (UPS). Amino acids coupled with insulin regulate the synthesis of protein within muscle tissue (Davis et al., 2010). It has been hypothesized that the mTOR pathway utilizes signals from both the amino acids and insulin including ribosomal protein S6 kinase 1 (S6K1) phosphorylation. Additionally, eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) affects mTOR regulation of protein synthesis and cell growth both up- and down-stream (Proud, 2006; Davis et al., 2010).

Both muscle fiber type and fiber number physiologically determine the growth potential of the animal, keeping in mind that muscle fiber type is plastic in nature. Fiber types I, IIa and IIb are commonly found within skeletal muscle of cattle, and vary based on metabolic characteristics. Type 1 fibers are small in diameter and are metabolically aerobic in nature, while type IIb fibers are large in diameter and are predominantly anaerobic in nature. Type IIa fibers are deemed intermediate or fast-oxidative and retain, as their name implies, an intermediate metabolism and protein accretionary properties in between type I and type IIb.

Fiber type and makeup directly impacts the post-mortem conversion of muscle to meat, impacting product quality indefinitely (Choi and Kim, 2009; Ryu and Kim, 2005). Adenosine

triphosphate (ATP) is primarily created via the glycolytic pathway utilizing glucose as stored glycogen. During the post-mortem conversion of muscle to meat, lactate accumulates within the muscle due to the cessation of blood flow. If glycogen content is not depleted, glycolytic fibers induce rapid post-mortem glycogenolysis and glycolysis which is characterized by decreased muscle pH. If this rapid pH decline is coupled with high temperature, then protein denaturation is increased resulting in decreased water holding capacity (WHC), pale lean color, and coarse lean texture (Joo et al., 1999). A study by Ryu and associates (2006) indicated that the increased concentration of type IIb fibers results in faster and more dramatic pH decline, resulting in higher lactate content and increased drip loss. In cases where the muscle fiber types are mixed, the pH decline is much faster than in muscles that exhibit primarily one fiber type (Lefaucheur, 2010).

Tenderness is also influenced by muscle fiber type and composition (Maltin et al., 2003). A high concentration of type II fibers are more susceptible to proteolytic degradation than muscles composed of type I fibers (Xiong et al., 2007). Higher calpain/calpastatin ratios have also been reported in type II and intermediate fibers than that found in type I fibers (Ouali & Talmant, 1990), therefore increasing the prevalence of type I fibers improves tenderness (Maltin et al., 1998).

Addition of new myonuclei is accomplished by satellite cell activation, recruitment, maturation and fusion. Originally defined in 1961 (Mauro et al.), quiescent satellite cells have their own transcriptome and reside between the basal lamina and the sarcolemma (Muir et al., 1965). New myonuclear accretion requires activation of the satellite cells, as well as proliferation and differentiation into myoblasts (Adams et al., 1998; Barton-Davis et al., 1999) that can then cause hyperplasia (ie; in the case of post-natal muscle damage and repair), or fuse

within existing myofibers causing hypertrophy (ie; muscle growth and protein accretion) (Carlson et al., 1983; Rowlerson et al., 2001).

Several transcription factors regulate skeletal muscle myogenesis, and belong to three main families or categories. The paired-box transcription factors (PAX 1-9) category represents a group that has roles in developmental processes such as vertebrate organogenesis (Mansouri et al., 1996; Buckingham et al., 2005). Pax3 and Pax7 have particularly important roles in somite and subsequent skeletal muscle growth and development. Nearly all myoblasts originate from cells that express Pax3, in addition to Pax7 during limb bud formation (Hutcheson et al., 2009). Pax7 expressing cells give rise to fetal myoblast as well as myocytes that contribute to adult myogenesis (Lepper et al., 2010). Absence of Pax3 or Pax7 results in formation of the myotome, but the primary and secondary myofibers fail to form (Relaix et al., 2004). Pax3 and Pax7 are also known to drive differentiation and determination of myoblasts both directly and indirectly (Collins et al., 2009; Sato et al., 2010). Seale and associates (2000) demonstrated that Pax7 is also used as a marker for satellite cell activity and is uniquely expressed in primary myoblasts, as well as quiescent satellite cells. Olguin and Zammit (2004) suggested that Pax7 regulates satellite cell propagation and renewal as well as specification of pluripotent progenitors.

The second family, also transcription factors, consists of myogenic regulatory factors (MRFs). These MRFs belong to the family of basic helix-loop-helix transcription factors involving two alpha-helices that are important for all segments of myogenesis, development, and maturation. All MRFs are responsible for regulating their own cognate gene transcription and translation rate as well as the expression of many other skeletal muscle specific gene actions including sarcomeric myosin. MyoD, Myf4, and Myf5 are crucial for the regulation of

myogenesis. Pax3 and Pax7 activate the MRFs which results in the proliferation and differentiation of skeletal muscle precursor cells (Relaix et al., 2005). Additional MRFs are responsible for anti-apoptosis actions as well as differentiation (Nabeshima et al., 1993). Knockout of any of these MRFs can cause reduced muscle mass, malformation and cell death.

The third major family of transcription factors is the MADS (MCM1, agamous, deficiens, and serum response factor) domain containing myocyte specific enhancer factor (Mef2, -a -b -c and -d). Transcription of Mef2s is mediated by MRF action (Wang et al., 2001; Dodou et al., 2003) and act on muscle specific gene promoters to mature the myocyte and result in fusion into new or with existing myotube (Steiner et al., 1999). Knock outs of Mef2s results in disruptions in myotube formation as well as sarcomere assembly.

Myogenesis is altered by dietary as well as environmental factors that the animal may be exposed to, such as; nutrient restriction, injury, and immunocompromise. Understanding the influence of these factors allows for substantial gain in livestock production techniques. As mentioned, commonly used extra-dietary tools include growth promoters such as melangestrol acetate (MGA), and metabolism modifiers like steroid implants (trenbolone acetate/estradiol) or beta-adrenergic agonists (ractopamine hydrochloride). Estrogenic implants increase the production of somatotropin (ST) and insulin-like growth factor-1 (IGF-1) which simulate bone and muscle growth. Androgens such as testosterone and trenbolone acetate (TBA) increase muscle IGF-1 receptors and stimulate increased accumulation of muscle protein.

Progestins (progesterone) increase digestive efficiency, nutrient partitioning and feed utilization (Gadberry et al., 2008). Pampusch et al. (2003) showed that cattle implanted with an estrogenic, androgenic, or combination implants showed statistically higher muscle IGF-1 mRNA levels between 12 and 26 days post-implantation. FDA approved beta-adrenergic

agonists are a feed additive that redirect the flow of nutrients from one priority to another. In this case, the nutrients are redirected to synthesize more muscle rather than fat or other body tissues. Ractopamine hydrochloride is a beta-1-agonist that increases the synthesis of lean muscle tissue with little to no impact on degradation of old tissue or fat stores (Garmyn & Miller, 2014).

Beyond the provision of a high-energy finishing ration to cattle, other dietary interventions are commonly employed such as the inclusion of ionophores, specifically rumensin (Goodrich et al., 1984) and rumen-protected amino acids (Hussein and Berger, 1995). These production practices allow for increased animal performance through very different modes of action. Implants are intended to increase body growth, specifically skeletal muscle and bone growth by increasing synthesis or anabolic efficiency. MGA is used in heifers to prevent reproductive cyclicity and partition nutrients towards growth. In contrast, beta-agonists increase the rate of protein synthesis, while mildly slowing the rate of breakdown to increase the overall accretion rate. Ionophores are intended to increase feed efficiency by buffering the rumen and altering the microbe population while rumen-protecting amino acids allow for the bypass of AAs to the small intestine where the animal can absorb and utilize it without interference from the rumen microbes. Many of these practices are coupled together to benefit from additive effects when it is economically feasible to do so.

During finishing, growth typically follows the path of a sigmoidal curve where the period of accelerated growth plateaus as the animal reaches maturity (Owens et al., 1993). During this maturation, the metabolism slows, and the animal becomes less and less efficient in growth and nutrient use. At this point, less nutrients are being used for hypertrophy of muscle for growth, and more are being partitioned to adipose tissue proliferation and development.

When this is coupled with a high plane of nutrition, as seen in commercial feedlot systems, the animal's intake will decrease. This process varies depending on breed, frame size, physiological maturity, and production system/environment, among other causes. These factors make reaching a "uniform" cattle lot at finishing challenging.

Conversion of muscle to meat

Beef quality involves many factors such as appearance, molecular metabolism, intramuscular fat presence, cookery, and overall eating characteristics such as flavor and tenderness (Bass et al., 2008). Post-mortem, a series of predictable changes via the transition of aerobic to anaerobic metabolism occurs. This can be altered by substrates present in the muscle at the time of harvest, such as glycogen. At the time of slaughter, the muscle utilizes glycogen reserves for energy. Without an intact circulatory system, metabolic acids are not cleared from the muscle and lactic acid builds up and causes the muscle pH to decline (Scanga et al., 1998). This decline in pH changes the structure of proteins within the muscle allowing degradation and some denaturation of the myofibrillar proteins. Some enzyme dependent post-mortem proteolysis occurs at this point. Many unfavorable product attributes may occur due to deviations in the normal rate of pH decline, as well as altered final muscle pH. For example, dark cutting is a quality defect due to a final post-mortem pH of greater than 6.8 caused by ante-mortem glycogen depletion (Scanga et al., 1998; Immonen et al., 2000). During the case of a dark-cutter, the lean color appears much darker red, with more blue pigment, making it less desirable to consumers. In addition, the water holding capacity (WHC) of the product is higher than that of normal beef. Conversely, a condition called pale soft and exudative (PSE) can be observed if the pH declines too quickly or reaches a final pH lower than 5.6 (Scanga et al., 1998). During this defect, the accelerated rate of pH decline and an increase in total acid, causes

denaturation of proteins and decreased WHC. Off-flavors or metallic flavors usually accompany this defect, in addition to nutrient leakage with the exudate (Lawrie, 1958; Grandin et al., 1997).

Sensory attributes of beef

Tenderness is considered to be the single most important attribute of beef product palatability according to consumers (Mackintosh et al., 1936; Morgan et al., 1991; Koohmaraie et al., 1995; Huffman et al., 1996), though it can be argued that safety and flavor are even more essential. That being said, proteolytic post-mortem tenderization has been a large topic of research driven by the capacity to optimize and provide consistency to the eating experience of consumers. In brief, the rate and extent of proteolysis via enzyme action has been deemed a major determinant of end-product tenderness (Oauli et al., 2006; Koohmaraie et al., 2006; Laville et al., 2009). Muscle calcium activated proteases (calpains) and cathepsins aid in the process of degrading troponin T and tropomyosin providing a more tender product (Koohmaraie et al., 1999; Lametsch et al., 2003; Hwang et al., 2005; Shibata et al., 2009). The proteasome complex is also considered the third proteolytic system involved in post-mortem tenderization (Koohmaraie and Geesink 2006). To be brief, I will only superficially discuss calpains as they can be inactivated by oxidation. Calpains are broken down into three subgroups: tissue specific, ubiquitous, and atypical (Sentandreu et al., 2002). The predominant peptidases responsible for post-mortem proteolysis of histidine-containing myofibrillar proteins are ubiquitous calpains (Delgadao et al., 2001; Sentandreu et al., 2002). Calpains such as μ-calpain and m-calpain, degrade desmin, tropomyosin, troponin T, troponin I, titin, nebulin and many others. Conversely, they do not degrade α-actin, α-actinin, or myosin heavy chain proteins (Huang and Forsberg, 1998).

Flavor is perceived from the combination of taste and aroma (Brewer, 2006; Maughan et al., 2012). Volatile compounds formed during cooking is a contributor to the flavor of meat (Mottram, 1998), in addition to carbohydrates, lipids, and proteins found within the muscle (Spanier et al., 1997; Mottram, 1998). Flavor can also be influenced by aging practices, temperature, diet of the animal, muscle location, marbling, and several other factors (Brewer, 2006). Hodges et al. (1974) found that off-flavors started to increase after 7 days of storage and are hastened by subject to retail display. Oxidation of lipids and proteins can produce volatile compounds, such as malonaldehyde, responsible for the incidence of rancid or off-flavors. A reduction in oxidation of lipid and protein preserves flavor profiles.

Juiciness is related to the liquid detectable during chewing of meat (Blumer, 1963), and plays a role in texture of the product. Juiciness can be affected by cooking method, marbling, protein denaturation, and other factors (Winger et al., 1994). During post-mortem proteolysis, the pH of the lean decreases as lactic acid is built up. As the pH approaches the isoelectric point at 5.25, the WHC is drastically reduced due to protein denaturation and the shortening of sarcomeres (Huff-Lonergan and Lonergan, 2015), which considerably impacts the consumers eating experience.

Color

The appearance and color of beef is the largest deciding factor as to whether or not a consumer decides to purchase the product (Dunsing et al., 1959; Jeremiah et al., 1972; Kropf et al., 1980; Gatellier et al., 2005; AMSA 2012). Increased color stability means increased probability of a consumer purchasing that product, making it more marketable (Wheeler et al., 1996). During retail display, many products are discolored due to oxidation, light exposure, and varied oxygen concentrations. Oxidation of lipid and protein cause discoloration and disruption

of the color producing agents within meat (Ma et al., 2010). Several antioxidants, namely vitamin A and E as well as carnosine (histidine-containing dipeptide), have been shown to improve the color stability of beef (Sanches-Escalante et al., 2001).

Heme containing myoglobin, hemoglobin, and cytochrome C are the major players in meat color, myoglobin being the most critical. The iron-containing heme molecules are able to form bonds with pyrrole nitrogen, one bond with a ligand (determinant of color), and the final bond with histidine; creating a total of six bonds (Mancini et al., 2005). Faustman et al. (1990) determined that three chemical forms of iron-ligand binding are responsible for color; oxymyoglobin (oxygenated), deoxymyoglobin (deoxygenated), and metmyoglobin (low levels of oxygen). Oxymyoglobin is due to binding with oxygen and yields a bright red cherry color, deoxymyoglobin is due to no binding with the ligand creating a dark purple color, and metmyoglobin is due to oxidization of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) and appears as a brown gray pigment.

Muscles can be classified based on color stability (McKenna et al., 2005). The accumulation of metmyoglobin allows researchers to categorize muscles into “very low”, “low”, “intermediate”, and “high” color stability through reflectance at different wavelength spectrums. Metmyoglobin reductase enzyme activity affects the rate of metmyoglobin accumulation and consequently, reductase activity to oxygen consumption rate determines color stability (Ledward, 1985; Mckenna et al., 2005). When oxygen consumption rate reaches its low point during retail display, color stability begins to decrease. In addition, Madhavi et al. (1983) found that increased metmyoglobin reducing activity was related to increased color stability. Muscles considered highly color stable typically have higher reductase activity, are resistant to oxidation of ferrous iron, and have lower rancidity rates (Reddy et al., 1991).

Metmyoglobin accumulation can be evaluated via the amount of surface reflectance at 572 nm to 525 nm with higher values exhibiting lower metmyoglobin formation (AMSA, 2012; McKenna et al., 2005). Madhavi and Carpenter (1993) found improved color stability for steaks fabricated on days 4 and 7 post-aging. At day 7, oxygen consumption rate reaches its plateau. McKenna et al. (2005) also showed that muscles with greater resistance to metmyoglobin formation, greater nitric oxide reducing ability, and lower oxygen consumption and penetration rates were considered "high" color stability and thus exhibited less oxidative rancidity than muscles with the converse characteristics.

Proteins and protein metabolism

In order to keep this literature review succinct, I will provide a very general overview of protein nutrition and metabolism. Proteins, and the balance of amino acids they are comprised of, are critical for mammalian processes such as muscle and organ growth, hormone production and action, general metabolism, cell signaling and transport, gene transcription and translation, and many other unlisted pathways. Animals receive protein through the diet in different forms; rumen undegradable protein (RUP), rumen degradable protein (RDP), and non-protein nitrogen (NPN). Once the protein sources reach the rumen, the RDP is broken down by the rumen microbes, repackaged into substrates for use by the animal (microbial protein-MP), and then transferred to the omasum and abomasum. Within the abomasum, acids and enzymes (pepsin) break down and denature the RUP and MP into large polypeptides and peptides. Those polypeptides and peptides then pass to the small intestine where proteases, such as trypsin, chymotrypsin, carboxypeptidase, and elastase, break down the polypeptides into smaller peptides and AAs. The AAs and peptides then cross the brush border into the lumen of enterocytes, where they are then transferred into the blood stream. Once in the blood stream,

these nutrients can be partitioned to different physiological purpose such as lactation support, and in this case, muscle growth. Excess nitrogen from the rumen is absorbed into the blood, detoxified by the liver, and can be recycled via urea back to the rumen, or can be excreted through urine via the kidneys.

Histidine, Anserine, and Carnosine

Histidine (HIS) is considered the third most limiting amino acid (AA) in finishing ruminants (Chalupa et al., 1973; Greenwood et al., 2000), especially in high-performing cattle. According to the National Research Council (NRC 2007) on the study of nutrient requirements of small ruminants, following methionine and lysine, histidine is the next most limiting amino acid in standard livestock feedstuff. HIS is encoded by the codons CAC and CAU and contains an amino group, a carboxyl group, and an imidazole side chain. Due to its chemical structure, HIS is positively charged at normal physiological pH. HIS is also closely related to formation of other physiologically important compounds such as histamine, important for immune and inflammation responses. Its chemical structure allows it to bind to the ligand in metalloproteins and thus, is an important part of catalytic sites in enzymes. HIS also has the ability to change from protonated to unprotonated states, allowing it to participate in acid-base catalysis.

Furthermore, HIS is converted to two metabolites within the muscle; carnosine and anserine. These metabolites provide antioxidant properties that may preserve color, prevent rancidity, prevent oxidation in general, and may be of human health importance. Both of these compounds are found in high concentrations in vertebrate mammalian muscle and brain tissues. Anserine and carnosine are found in higher concentrations in fast-twitch muscles (Type II) than in slow-twitch muscles (Type I) (Bump et al., 1990; Dunnet et al., 1997) which may indicate that more of these antioxidants may be formed and stored through muscle fiber hypertrophy.

Thus, anserine is found in higher concentrations in poultry tissues than in mammals. Anserine and carnosine are antioxidative in nature as they are zwitterions that contain no charge, meaning that they can scavenge reactive oxygen species and prevent oxidation. Due to these attributes, they may prevent oxidation of chromophores (improving color), and lipids thus decreasing rancidity, off-flavors, and finally these functional nutrients may be beneficial to human health. Carnosine, one histidine-containing dipeptide, is biosynthesized directly from the constituent amino acids, histidine and β-alanine and is catalyzed by the enzyme carnosine synthetase present in muscle (Winnick & Winnick 1959).

Similarly, another histidine-containing dipeptide, anserine, can be biosynthesized via methylation of carnosine involving the donor adenosyl-methionine and a specific N-methyl transferase (McManus 1962), also present in skeletal muscle. Carnosine has been described by O'Dowd et al. (1996) as having a physiological role in smooth muscle contraction augmentation when coupled with zinc. Anserine and carnosine have many other physiological roles as well including; activation of myosin ATPase, regulation of Ca^{2+} and sensitivity, excitation-coupling of muscle contraction, protecting proteins from oxidation and glycation, pH buffering, and some control of autonomic nerve action. Carnosine is thought to be transported across the brush border membrane with the help of the peptide transporter 1 (PEPT1) in humans, and thus, may be a good source of nutrient and beneficial for human health (Kubomura et al., 2009), though serum carnosinase does catabolize some. Anserine is absorbed and broken down in humans by serum anserinase (similarly to carnosine by carnosinase), indicating that it may also be a source of functional nutrient (Kubomura et al., 2009). These studies indicate that dietary supplementation alone may not allow significant increases in plasma carnosine and anserine,

but may still provide the substrates for further synthesis within the body tissues such as the brain and muscle.

Batrukova et al. (1996) found that the metabolite, carnosine decreases the rate of Ca^{2+} accumulation by the heavy fraction of the sarcoplasmic reticulum from skeletal muscle due to the ability of carnosine to induce rapid calcium release. Anserine, the derivative of carnosine, also induces rapid calcium release which increases the sensitivity of the Ca-release channels to their activators (AMP, Ca^{2+}, Caffeine, and etcetera). This indicates that the metabolite content of skeletal muscle dictates in part, the sarcoplasmic reticulum Ca-channel activity, and thus, increased muscle metabolite content can increase muscle contraction induction efficiency (Batrukova et al. 1996). While unproven, these metabolites could impact the activation of calpains since they are calcium dependent.

In addition, anserine and carnosine function as proton buffers over the physiological pH range aiding in the rapid healing and re-modeling of the skeletal muscle environment (Bate-Smith, 1938; Davey, 1960), and limiting mitochondrial damage in high metabolic states. Dunnett & Harris (1997) indicated that the diffusion of protons into muscle fibers during anaerobic exercise periods is in part carried out by anserine and carnosine. This acid-base balance is essential for muscle performance and growth in terms of the ability to transport oxygen via the cardiovascular system. In muscle increased availability of histidine results in the increased synthesis and storage of antioxidant metabolites namely; anserine and carnosine.

Histidine as it affects growth and efficiency

According to Kasakoa and associates. (2004), 50 g L-histidine supplementation per kg of feed suppressed food intake and fat accumulation in a lab strain of rats by activation of histamine neurons. Furthermore, it was seen that depletion of neuronal histamine within the

hypothalamus was correlated to obese phenotypes. A study by the same group indicated a negative correlation between energy intake and histidine per protein intake, thus showing that histidine supplementation suppresses appetite by activating histamine neurons (2002). Masaki et al. (2001), also reported decreased adiposity by upregulating mRNA expression of uncoupling proteins (UCPs) via the histamine neuron system. This upregulation led to sustained increases in serum leptin that is involved in energy homeorrhesis by inhibiting neuropeptide-Y which then provides a satiety signal, increasing insulin levels, decreasing appetite, and increasing physical activity in favor of muscle growth (Morton & Schwartz, 2011).

HIS is known to be a limiting AA for growing cattle (Chalupa et al., 1973; Greenwood & Titgemeyer, 2000). Previous data indicated that 50g/hd/d rumen protected histidine to growing beef steers for 129 d pre-harvest resulted in a tendency towards increased ADG (Thornton et al., 2015). McCuistion et al. (2004) showed that supplementation in diets deficient in metabolizable protein with rumen protected HIS improved overall AA and a protein status of the animal, thus improving protein deposition in growing cattle. In a study by Ma et al. (2010), finishing pigs were supplemented with carnosine for eight weeks indicating that there was no difference in growth traits, but the HIS utilization between animals was variable. Interestingly, Watanabe et al. (2004) reported that histidine, anserine and carnosine levels vary based on breed type and age of beef cattle showing a drastic increase in histidine and anserine, as well as a decrease in carnosine when animals exceeded 25 months of age. In addition, Japanese Black cattle had higher anserine and lower carnosine concentrations than Japanese Shorthorn and Japanese Holstein cattle. While anecdotal at this stage, it seems plausible that these antioxidants may contribute to some aspects of variability between differing species and breeds of cattle under various production systems. Furthermore, male rats (Bassil et al., 2007)

and catfish (Farhat, 2013) supplemented with dietary histidine at 5% of diet and 9.4 g per kg of diet exhibited decreased feed intake by forty-two and forty-nine percent respectively.

Histidine effects on production

It has been proposed that histidine is the first most limiting amino acid (AA) in lactating dairy cows fed a ration low in plant protein (Kim et al., 1999) and that in situations when microbial protein production is reduced, histidine deficiency limits milk production significantly (Lee et al., 2012b). Lee et al. (2012) found that histidine was a limiting amino acid in dairy cows fed a diet low in metabolizable protein (MP). Supplementation with rumen protected methionine and lysine diminished the decreased DMI and milk yield effects seen in the deficient cows, while histidine supplementation eliminated the depression in production completely while increasing milk protein concentration. In addition, a sharp decrease in plasma histidine concentrations was reported in cows fed diets deficient in MP, and histidine supplementation reversed this effect. Lee et al. (2012) found that in cows supplemented with rumen-protected histidine, milk yield was increased and overall milk protein content was increased in comparison to methionine and lysine supplemented cows. These findings indicate that HIS supplementation in rapidly growing beef cattle limited by HIS availability could increase protein synthesis and accretion within the lean muscle tissue.

Histidine effects on product

Lipid oxidation is a major cause of decreased meat quality in terms of flavor, color, and shelf-life (Gray & Pearson, 1987). Consequently, the products of fatty acid oxidation are associated with rancid or off-flavors (Gray & Pearson, 1994). Oxidants interact with unsaturated fatty acids resulting in free radicals and oxidative chain induction in muscle systems at the membrane level (Labuza et al. 1971; Ashgar et al. 1988). This rancidity can be measured

in the form of malondialdehyde by performing a thiobarbituric acid reaction assay (TBARS) according to the methods of Tarladgis et al. (1960). According to Greene and Cumuze (1981), oxidized flavor in beef was detected over a TBARS value range of 0.6 tp to 2.0 tp when the samples were packaged and subjected to simulated retail display. In addition to this, it is known that the incidence of off-flavors increases with time as a consequence of autoxidation of membrane phospholipids (St. Angelo et al., 1987), especially between days 4 and 9 of retail display (Campo et al., 2005). It was also reported that carnosine supplementation decreased the presence of oxidative products within the rat skeletal muscle, but did not increase carnosine concentration (Chan et al., 1994b).

Post-mortem muscle changes during rigor mortis are accompanied by increased oxidation of myofibrillar proteins resulting in the conversion of amino acids such as histidine to carbonyl derivatives (Levine et al. 1997) which have the ability to decrease the functionality of proteins resulting in water-loss and purge of the product (Xiong & Decker, 1995). Both u-calpain and m-calpain, enzymes responsible for pre- and post-mortem protein breakdown and turnover, contain high levels of histidine and SH-containing cysteine residues at their active sites making them vulnerable to oxidation (Huff-Lonerghan & Lonerghan, 2005). The oxidation of the calpain enzymes causes inactivity and thus, a decrease in protein breakdown/turnover increasing shear force values creating a less tender product.

An additional benefit associated with supplemental HIS relates to the potential to enhance beef color and color stability in accordance with deposition of histidine metabolites (anserine and carnosine). Anserine and carnosine are known antioxidants (Boldyrev et al., 1988; Wade et al., 1998; Thornton et al., 2015) which are found in reasonably large quantities within vertebrate skeletal muscle (Crush 1970). A study in pigs verified that elevated carnosine

in muscle improved the redness of the meat, decreased purge and increased the anti-oxidative capacity in the muscle (Ma et al., 2010).

Kopec et al. (2012b) reported an increase in breast muscle anserine and carnosine when growing broilers were supplemented with dietary histidine. In addition to increased antioxidant status, the supplemented broilers also increased in body weights, ADGs, feed conversion indices, breast muscle protein content, and overall histidine content of the muscle.

Dunnett and Harris reported that dietary supplementation of histidine resulted in increased carnosine and anserine in the top sirloin or *gluteus medius* (GM) muscle (2008). Thornton et al. supplemented 40 finishing steers implanted with Revalor® S (medium potency steroidal implant) for the final 129 d of finishing using a Calan gate system. The steers were fed a TMR feedlot diet and top dressed with 50 g/hd/d rumen protected HIS. Thornton observed trends for increased anserine and carnosine deposition in the *longissimus dorsi* (LD) muscle of HIS supplemented steers (2015). As mentioned, both anserine and carnosine are antioxidants, that can stabilize chromophores in meat to improve color and stabilize lipids to improve flavor and stabilize proteins to enhance juiciness through improved water holding capacity (WHC). Increased antioxidant content may also reduce the incidence of off-flavors within the product, thus leading to a more consistent eating experience.

A feeding experiment by Haug et al. (2008) involving histidine supplementation to broiler feed, they measured a significant increase in concentration of the histidine containing dipeptides; anserine (β-alanyl-N-methylhistidine) and carnosine (β-alanyl-L-histidine) in broiler breast muscle. Anserine and carnosine are antioxidants that can stabilize chromophores in meat to improve color and stabilize lipids to improve flavor and stabilize proteins to enhance juiciness through improved water holding capacity. Thornton et al. (2015) published data

showing that steers receiving histidine for 129 d pre-harvest had increased a* ($P = 0.004$) and b* ($P = 0.05$) color values during the 9 d simulated retail display (Thornton et al., 2015). Evaluation of the top sirloin following AMSA color evaluation guidelines demonstrated that there was less ($P \leq 0.01$) browning, discoloration and surface discoloration in the steaks from steers receiving histidine. Moran et al. (2012) showed that treatment of fattening lambs with carnosic acid delayed lipid oxidation and improved color attributes of the *gluteus medius* muscle. Additionally, meat texture was equally improved and decreased cholesterol oxidation in the same product.

Antioxidants (carnosic acid) derived from compounds such as rosemary extract (Sanchez-Escalante et al., 2001) reduce discoloration when applied to the surface of meat products (Djenane et al., 2002). Rosemary powder (1000 ppm) and rosemary with ascorbic acid (500 ppm) were applied to ground beef patties and inhibited the formation of metmyoglobin as well as lipid oxidation (Sanchez-Escalante et al., 2001). Other forms of treatment include ascorbic acid, taurine, vitamin C, vitamin E, and carnosine. By increasing the carnosine content within the muscle, we may be able to improve color without the added step of treating the product post-fabrication, eliminating cost and potential contaminants or health risks. This increase in color stability and consumer acceptability will improve consumer perception, allowing the potential for increased sales and consumption of beef products.

Histidine's potential beneficial effects on human health

Oxidation is a main factor affecting human aging and oxidative diseases (Wu et al., 2004). As an added benefit of the healthful benefits of beef, dietary carnosine and anserine ingestion by humans has been shown to increase intense human exercise performance (Maemura et al., 2006). Further, published research has indicated that carnosine may be

neuroprotective (Min et al., 2008) and protect DNA from oxidative damage (Hsieh et al., 2002). While these associations remain anecdotal one can imagine that it may be plausible that marketing of beef that is innately higher in content for these antioxidants may provide an added economic value. Waagbo et al. (2010) showed that histidine supplementation in Atlantic salmon in seawater decreased the incidence of cataract development by prevention of oxidation and histidine deficiency, though similar research has not been evaluated in humans. Liu et al. (2008) showed that supplementation with histidine and carnosine transiently upregulated the mRNA expression of IL-6 and TNF-alpha, cytokines known to be involved in the inflammation and healing processes. Perhaps the anti-inflammatory and antioxidant effects of histidine and carnosine may be used in human health in terms of treatment of chronic liver injury and other disease associated with dysregulation of the inflammatory process. The advantages of all of these potential health benefits may become exceedingly relevant given the continual rise in age related dementia, Alzheimer's and metabolic disorder in humans.

Research Focus

The focus of this research is directed towards understanding the regulation of skeletal muscle growth in finishing beef cattle with an emphasis on production performance and product quantity and quality of beef. In the beef cattle sector, lean skeletal muscle tissue is of utmost importance, and thus, understanding the nuances of skeletal muscle growth, acquisition, and metabolism will allow for improvements in the beef industry in terms of production, product quality, efficiency, and the overall sustainability. By using a natural nutritional intervention, we may be able to improve growth, lean color, eating experience, shelf-life, and the healthful attributes of beef. A short duration pre-harvest supplementation with rumen-protected HIS falls in line with conventional beef production practices while providing a more desirable product at

a potentially lower input-cost. Not only does this benefit the producer in terms of the bottom line, the consumer is directly impacted by way of eating experience and the healthful attributes of the purchased product. This is will require development of a cost-effective source of HIS, that is outside of the scope of this research but may include supplementation of dietary sources naturally high in histidine into finishing rations.

Development of hypothesis I

Historically, feed products account for 70-90% of the cost incurred by beef cattle producers. Increasing prices and decreasing availability of feedstuffs has made it more imperative that the animals produced are efficient in utilizing feed nutrients for the intended physiological system, in this case, growth. However, this increase in efficiency and growth must not negatively impact product quality. Producers have placed more emphasis on acquiring affordable feed ingredients that meet the macronutrient requirements (ie: crude protein, crude fiber, NDF, ADF, etc.) of the animal to sustain growth, rather than balance the ration based on amino acid content and the implied limitation of protein synthesis due to shortage of one or more essential amino acid in the animal's diet. Histidine is known to be the third most limiting AA for growing cattle (Chalupa et al., 1973; Greenwood and Titgemeyer, 2000) behind lysine and methionine. Furthermore, previous studies have shown that supplemental HIS improves dairy cattle performance (Vanhatalo et al., 1999; Huhtanen et al., 2002; Lee et al., 2009). Thornton et al. (2012) showed that supplementation in beef cattle at one dose, 50 g/hd/d, for the final 129 d of finishing tended to improve ADG. However, no other published research has evaluated dietary histidine use in beef cattle. In order to condense the supplementation period and evaluate dose dependency, we hypothesized that *supplemental dietary histidine at two doses, 50 g/hd/d and 100 g/hd/d, for the final 60 d of finishing increases muscle growth in*

growing cattle. In addition, we utilized a more aggressive steroid implant (Revlor-XS) to simulate what is used in conventional beef production, as well as evaluate the effects of HIS within a period of increased growth where the AA profile of the animals may be limited. The supplemental period selected mimics the final sort of cattle into their final phase counterpart groups (about 55 days pre-harvest). This allows for ease of implementation for the producer.

Development of hypotheses II & III

The visual appearance and color of fresh meat is thought to be the most impactful deciding factor in purchasing beef at retail (Dunsing et al., 1959; Jeremiah et al., 1972; Kropf et al., 1980; Cornforth, 1994; Gatellier et al., 2005). Discoloration of the product may occur during retail display which may deter a consumer from purchasing it. This discoloration varies by cause and rate, effecting the value of the product. Lipid and protein oxidation affect the stability of color as well as the palatability of the product by way of flavor composition and profile (Ma et al., 2010). It is known that antioxidants often administered as an injectable, such as vitamin E (Faustman et al., 1998) have been shown to prevent oxidation and thus improve the color stability of beef products. Carnosine and anserine (histidine-containing dipeptides) have also been shown to possess antioxidative properties that may reduce the oxidation and thus discoloration of the product. Thornton et al. (2012) improved color scores in the *gluteus medius* muscle through histidine supplementation for the final 129 d of finishing. Mckenna et al. (2005) classified the gluteus medius as a "moderately color unstable" muscle and the *longissimus lumborum* as a "color stable" muscle. The comparison of the two muscles may indicate the efficacy of histidine supplementation across the entire carcass. The ability to reliably improve sensory and product characteristics of beef is an indispensable asset and may boost the value of each carcass. Improving growth and feed efficiency has the ability to decrease

input costs allowing for the producer to demand a higher profit. Finally, lower input costs allow for a lower price commanded by the retailer, thus a more affordable, higher quality product for the consumer. Because there is no other research evaluating dosage and color effects in multiple muscles, we hypothesized: *dietary histidine supplementation will improve color and color stability of the gluteus medius and longissimus lumborum in beef cattle provided supplemental histidine by increasing deposition of natural anti-oxidant by-products; anserine and carnosine.*

As mentioned, oxidation of lipid causes formation of compounds that negatively impact flavor profiles (Labuza et al., 1971; Tarlagdis et al., 1960). Oxidation of structural myofibrillar proteins increases water loss and purge causing decreased WHC and juiciness (Crush et al., 1970; Boldyrev et al., 1988; Lonergan et al., 2001; Ma et al., 2010). No published research has evaluated histidine supplementation in beef cattle and its effects on sensory attributes of the product. Due to the antioxidative properties of anserine and carnosine, we hypothesized: *supplemental dietary histidine will improve sensory attributes such as flavor, WHC, juiciness and consumer acceptability of the gluteus medius and longissimus lumborum.*

Additionally while not yet entirely proven, anserine and carnosine are neuroprotective in nature and if provided in the human diet may have the benefits of reducing Alzheimer's, dementia, and other neurodegenerative diseases. This allows for the expansion and marketability of a more healthful beef product and could increase beef consumption within the US and internationally.

CHAPTER 2

An Examination of the Effects of Dietary Rumen-Protected Histidine Supplementation on Finishing Beef Cattle Growth, Carcass, and Meat Quality Parameters

Introduction

In March 2014, the USDA reported that per-capita annual beef consumption has fallen almost 23 lbs. since 1985. It is expected to fall an additional 4.5 lbs by the year 2023. While the domestic market will still remain an important component of economic sustainability, it is becoming imperative that we expand "beef" export to regions of the world where beef consumption is increasing as an essential component of sustainability of the US beef production industry. Increases in feed costs and competition for available high quality protein sources may be causing producers to unknowingly limit their growing livestock of essential dietary amino acids (AA) fed to livestock. This prevents the animal from reaching true growth potential. Consumer acceptability of the product in terms of flavor profile, color, tenderness, juiciness, and the presence of off-flavors directly impacts the consumption and sale of beef products in the U.S., as well as on export markets.

Furthermore, shipping duration and day from harvest to marketing may rise over the next decade due to increased exports. Therefore, one product quality parameter; color stability, may become a quality trait of increasing importance. Optimal color profiles may help to ensure that US beef has the most appealing marketable appearance at new and developing export markets, relative to other competing beef export nations, such as Brazil, Canada, Argentina and Australia. Inevitably, the concept of RP-HIS supplementation has the potential to improve beef quality and consumer appeal through a cost effective nutritional pre-harvest intervention. Pre-harvest strategies such as this are designed to enhance livestock performance via nutrient alteration, and have the ability to favorably alter the quality aspects of beef. Consequently, this

route of product enhancement is perceived as natural and is the most widely accepted producer intervention by consumers (Grunert, 2011).

Skeletal muscle accounts for 40-60% of lean body mass in beef animals and plays important roles in metabolism, systemic physiology, homeorrhesis, and locomotion. In addition to the attainment of physical motion, skeletal muscle facilitates glucose storage (in the form of glycogen), thermoregulation, water regulation, and plays crucial roles in energy metabolism. Lean muscle accretion and growth also provides the template for marketable beef product consumed across the globe as a high-quality source of protein and other nutrients. Thus, the quantity and quality of meat is completely dependent upon that animal's ability to accrete striated muscle mass efficiently in a relatively short time-period. Understanding the concepts of skeletal muscle physiology is important for both the growth of lean muscle, as well as the post-mortem conversion of muscle to meat and the associated changes in metabolism that impact product quality and product marketability.

Materials/Methods

Animal care

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) as required by federal law and University of Idaho policy.

Human subject use in consumer panel

The University of Idaho Institutional Review Board determined this project to be exempt (Protocol 2015-41) (Appendix A).

Animal treatment protocols

Forty-eight weaned (aged 8-11 months) Sim-Angus crossbred steers of known lineage were obtained from the University of Idaho's Nancy M. Cummings Research and Extension

Education Center in Carmen, ID. The cattle were transported to the University of Idaho's Monson Barn (Moscow, ID) where they remained for the duration of feeding. The cattle were unloaded in the evening of December 16, 2015, bunk fed and weighed bi-weekly until time of sorting. They were fed a total mixed ration (TMR) (Appendix B) twice daily (07:00 and 16:00) to reach maximum intake, both feed and water access were *ad libitum*. All intermediary ration formulations are attached (Appendix C). Post-arrival, all cattle were vaccinated according to label with Inforce 3 (IBR, BRSV, PI_3), One Shot (BVD, M. hemolytica), UItrabac 8-Way, and Bovi-Sheild 5 + One Shot, and were de-wormed using Dectomax (all animal health products excluding Ivomec and Revalor®-XS were provided by Zoetis). The cattle were treated with Ivomec thirty days later to resolve any liver fluke infestations. 15 days after arrival, the cattle were moved inside the Monson Barn, randomized by body weight, assigned a treatment group (control: no RP-HIS, low HIS: 50 g/hd/d RP-HIS, high HIS: 100 g/hd/d RP-HIS), and sorted into pens (6 hd/pen across 8 pens) to begin Calan Gate (Type A System, American Calan, Northwood, NH) training. Each pen of animals consisted of two animals of each treatment. Pens had a concrete floor and steers were bedded with a wood shaving/wood chip mixture.

The training process was made up of four segments: 1) the gates were tied open (about 2 weeks) until the cattle were feeding consistently from the experimental bunks; 2) the gates tied only half way open; 3) gates closed while preventing locking of the gates allowing the steers to push the gates open freely; and 4) the steers were assigned gates and fitted with collars/transponders, and the locking mechanism was engaged. Each of these phases allowed for the cattle to become acclimated to the Calan gate feeding system and become trained to use of each assigned gate/feed bunk. Due to low light levels inside the barn, the steers were supplemented subcutaneously with vitamins A, D, & E (5 ml/hd) 85 days prior to administration

of HIS supplement. This was not ideal as vitamins A and E are known antioxidants, but was necessary to prevent deficiency. The steers were implanted 40 d prior to supplementation with Revalor®-XS (Merck Animal Health, Summit, NJ). Revalor-XS is rated for 120 d efficacy and was used to allow for peak gain and substance release during RP-HIS supplementation. Individual intakes were recorded by pre-weighing feed for each animal and adjusting according to bunk score. Orts were evaluated by scooping each bunk and brushing with a broom and dust pan to obtain weight of unfinished feed. Both intakes and orts were recorded beginning 20 d prior to supplementation, and through the duration of feeding. Eleven random grab samples (Appendix D) of the ration were obtained from the combined orts from that day and dried to determine percent dry matter in drying ovens at 65˚C for 96 hours and then analyzed by Cumberland Valley Analytical Services, INC (Waynesboro, PA). Upon initiation of the trial, the morning feeding was top-dressed with individually pre-measured HIS based on prior studies indicating effective dose (50 g/hd/d) and a high histidine level (100 g/hd/d). Three treatment groups were utilized with 16 animals in each group, Control (no HIS), low HIS (50 g/hd/d HIS), and high HIS (100 g/hd/d HIS). The HIS product (Balchem Corporation, New Hampton, NY) contained 55-60% bioavailable HIS and has been shown to be highly rumen-protected and bioavailable in the ruminant (Little, 1975; Patton & Parys, 2012). Cattle were weighed prior to the morning feeding on a bi-weekly basis to limit gut fill and improve accuracy of the measurement. At the conclusion of the feeding period (60 d of HIS administration), the cattle were weighed a final time, and transported to Washington Beef (Toppenish, WA) to be harvested. No animals were excluded for health reasons or death. All animals treated for cases of morbidity were recorded in a sick log (Appendix I) and were evaluated to prevent use of outliers.

Carcass data collection

Hot carcass weight was recorded by Washington Beef, LLC (Toppenish, WA) and carcass data were acquired by two trained personnel from the University of Idaho. Rib eye area (REA), kidney, pelvic and heart fat (KPH), marbling score, quality grade (QG) and final yield grade (YG) were determined approximately 24 h after harvest. USDA carcass grading data were also determined for each carcass by Washington Beef via the VBG2000 Vision Camera. The carcass data taken by UI personnel was used for data analysis.

Product procurement and steak preparation

Forty-eight h post-mortem, one complete top sirloin (IMPS #184) and one complete strip loin (IMPS #180) was vacuum packaged and obtained from each carcass and transported to the University of Idaho Meat Science Laboratory. The sirloins were aged 14 d post-mortem and strip loins were aged 21 d post-mortem in vacuum package prior to being cut into steaks. Upon conclusion of the aging period, the sub-primal was removed from the vacuum package and weighed. The anterior end of the strip loins and top sirloins were prepared by removing a 2 cm-thick segment, and subsequently four more 2.54 cm-thick steaks were removed perpendicular to the long axis of the longissimus. From anterior to posterior, the steaks were used as follows: color analysis of the *longissimus lumborum* and *gluteus medius* (Hunter MiniScan EZ, Restin, VA); consumer taste panel; Warner–Bratzler shear force (WBSF) (GR Manufacturing, Manhattan, KS); and high performance liquid chromatography (HPLC) metabolite analysis (Waters e2695 and a Waters 2998 photodiode array detector, Milford, MA, USA). Only the *longissimus lumborum* (LL) and *gluteus medius* (GM) were evaluated. The LL and GM muscles were selected for their known differences in color attributes (Renerre, 1984). Steaks used for retail display were packaged in Styrofoam trays with oxygen permeable PVC

overwrap (Koch Industries, Inc. Wichita, KS) and were transported to the UI Food Science Lab for retail display, along with the steaks intended for cookery and WBSF (not packaged for retail display). The two steaks intended for Taste Panel and HPLC analysis were vacuum packaged and stored at -20˚C until time of use.

Color evaluation

All steaks were displayed in a glass retail display case (Model GDM-69, True Manufacturing Co., O'Fallon, MO) at 2 °C. The display case was equipped with natural white Hg 40 W lights with an average light intensity of 408 lux. Temperature within the display case was monitored and recorded daily via four mercury thermometers spaced throughout the case. Steaks were allowed to bloom for a minimum of 60 minutes prior to initial color readings. Instrumental color measurements were taken in duplicate for each steak using a Hunter MiniScan EZ (Restin, Virginia). The Hunter MiniScan was equipped with a 25 mm-diameter measuring area and a 10° standard observer. The instrument was set to illuminant A to allow more emphasis on red color, and Commission International de l'Eclairage (CIE) L* (lightness), a* (redness), and b* (yellowness) values were recorded in duplicate from each muscle. The machine was calibrated before each day of readings by measurement against black and white tiles. Each measurement surface was selected avoiding large marbling or connective tissue flecks, and well away from the edge. Measurements were taken in duplicate overtop of the oxygen permeable overwrapping with the steak in a Styrofoam tray on days 0, 1, 3, 5, 7, and 9 of display and the values were averaged for statistical analysis.

Steaks were also subjectively evaluated for oxygenated lean color, discoloration (1 = none; 5 = extreme), amount of browning (1 = no evidence of browning; 6 = dark brown), surface discoloration (0% = no discoloration; 100% = total discoloration), and color uniformity (1 =

uniform; 5 = extreme two-toning) by trained evaluators according to the Meat Color Measurement Guidelines (AMSA, 2012). The first pair of measurements represented d 0 of retail display. Subsequent color measurements were taken on days 1, 3, 5, 7, and 9 of retail display. The steaks were rotated daily according to uniform protocol to avoid affects due to location and temperature. Hue angle was calculated using the arctangent of (a*/b*) to indicate the vividness off the color profile. It is thought to be a good indicator of what may be visually observable by consumers.

Retail fluid loss

Each steak was weighed prior to packaging in a Styrofoam tray with oxygen permeable overwrapping with no drip paper for retail display and weighed at the end of retail color analysis (9 d) to determine percent retail fluid loss. The results were reported as percent retail fluid loss.

Cookery

Steaks for cookery were weighed and cooked on open-hearth broilers to an internal temperature of 40°C, then turned and cooked to a final internal temperature of 71°C. Temperature was monitored with hypodermic temperature probes (Omega Engineering Co., Stamford, CT) coupled with a 12-channel scanning thermocouple thermometer (Digi-Sense, Cole-Parmer Instrument Co., Vernon Hills, IL). Cooked steaks were weighed to determine percent cooking loss, and allowed to cool to room temperature.

Warner-Bratzler Shear Force

Room-temperature steaks were mechanically cored using a drill press-mounted coring device. Six cores (1.27 cm diameter) were mechanically removed parallel with the muscle fiber orientation. Shear force was determined by shearing each core once (200 mm/min) in the center using a Warner-Bratzler shear machine (GR Manufacturing, Manhattan, KS). The average

WBSF of six cores was reported.

Lipid oxidation

Lipid oxidation was evaluated for each sample (n=96, 48 of each muscle type) using a thiobarbituric acid reactivity assay (TBARS). Samples (approximately 2 g each) from each steak were collected at day 0 and day 9 of simulated retail display. This was done following the protocol Appendix O of the Meat Color Measurement Guidelines, provided in Section XI (AMSA, 2012). The tail end (about 1 cm) of each steak as well as the lower surface was discarded before samples were taken while avoiding the edges.

pH

Muscle pH was measured immediately before cooking for taste panel analysis. A portable pH meter (Seven2Go pro, Mettler Toledo, Woburn, MA) equipped with an InLab SolidsPro puncture-type electrode was used to measure pH of the LL from the anterior end of the strip loin and the GM from the anterior end of the top sirloin. The pH meter was calibrated using standard pH 4.0 and 7.0 buffers.

Consumer taste panel analysis

Two separate consumer panels were conducted, one for each muscle, at the Washington State University Sensory Evaluation Facility. Panels included top sirloin steaks (GM) and strip steaks (LL). For consumer panel analysis, steaks were thawed overnight at 4 °C and subsequently cooked as described for cookery analysis. Samples were kept warm until serving in covered cups labeled with a three-digit random number. A panel of consumers (n = 72 per muscle) evaluated cooked steaks from each treatment group for overall acceptability, flavor, tenderness, and juiciness. The panelists scored each sample using a 9-point scale (9 = like extremely, extremely tender, extremely juicy, and like flavor extremely, respectively; 1 =

dislike extremely, not at all tender, extremely dry, and dislike flavor extremely, respectively). Consumers were also asked if they could detect an off flavor, what trait they liked most/least (if applicable), and if they would be willing to purchase the product. Six 1.27-cm × 1.27-cm × steak thickness cubes were obtained from each steak using a cutting die. Each consumer evaluated four samples from an incomplete block design, one from each treatment group and one rotating sample. A copy of the evaluation form is attached (Appendix E).

Preparation for muscle extract metabolite composition

The steak intended for HPLC metabolite analysis was thawed overnight at 4˚C in preparation of sample grinding. One approximately 5 g sample was cut from the tail end of the LL and GM and snap frozen in liquid nitrogen. The tail and edges of the product were discarded (approximately 1 cm). The tissue samples were ground using a mortar and pestle under liquid nitrogen and dry ice, and approximately 250 mg of wet tissue was weighed into 2 ml conical bottom centrifuge tubes (5 tubes for each steak). Amino acids were extracted following the procedures of Aristoy and Toldra (1991). One mL of a 0.01 N HCl solution was added to the weighed tissue tubes. The tissue was homogenized using a Retsch Bead Homogenizer MM301 (Retsch, Newtown, PA, USA), for 5 × 20 s at 25 Hz. Samples were then centrifuged at 4˚C for 20 minutes at 10,000 g, at which point the supernatant was filtered through glass wool into a clean tube. The samples were then stored at -80˚C until deproteinized.

Deproteinization and derivatization of muscle extracts

The filtered samples were deproteinized following the methods of Aristoy & Toldra (1991). Generally, 50 µL of hydroxyproline 0.325 mg/mL (internal standard) and 750 µL of HPLC-grade acetonitrile were added to 250 µL of thawed filtered sample and allowed to stand at room temperature for 30 minutes. Samples were then centrifuged at 10,000 g for 15 minutes

at 4˚C. 200 µL of the centrifuged samples were then immediately derivatized according to the methods of Bidlingmeyer et al. (1987). Samples were dried at 38˚C under vacuum and nitrogen in a Pyrex culture tube using a Waters Pico Tag Work Station (Waters, Milford, MA, USA) with 20 µL of a methanol-1M sodium acetate-triethanolamine (TEA) (2:2:1) solution. 20 µL of a methanol-water-triethanolamine-phenylisothiocyanate (PITC) (7:1:1:1) solution was then added to the culture tube and allowed to stand for 20 minutes at room temperature. The samples were then dried again under vacuum and nitrogen. 100 µL of a 5mM sodium phosphate pH 7.6 with 5% acetonitrile was then added to reconstitute the dried sample. They were then immediately analyzed using HPLC. All solutions for derivatization were prepared fresh daily.

HPLC

Derivatized samples were analyzed on a Waters e2695 separations model equipped with auto-sampling abilities and a Waters 2998 photodiode array detector (Waters Corp, Milford, MA) set to 254 nm. A Waters Symmetry® C18 3 x 150 mm (5µm particle size) column was used. The column was heated to 40˚C ± 1˚C and the sample was cooled to 5˚C ± 1˚C within the holding space. The HPLC method was adapted from Aristoy & Toldra (1991). Two eluents were used as solvents: (A) 0.14 M sodium acetate containing 0.5 mL/L of triethanolamine at pH 6.4 with 5% acetonitrile; (B) acetonitrile/water 60:40. The flow rate was set to 0.8 mL/min and a gradient was performed (Appendix F) to allow for linear change and wash for 8 minutes between samples to re-equilibrate to 10% B over 20 minutes before new injection. 20 µL of sample were injected into the system and then Waters Empower® software was used for metabolite detection. Four different standards were utilized; hydroxyproline (internal standard), L-anserine, L-carnosine, and L-histidine (Sigma Aldrich, St. Louis, MO). All samples were run in duplicate and quantified based on standard curve values. All standard curves had an R^2 of at

least 0.95. Free anserine, carnosine, and histidine values were reported as mg/g wet tissue weight corrected to 100% for internal standard recovery.

Statistical Analyses

Body weights were utilized to provide estimates of dietary influence on average daily gain, and individual intakes were used to calculate feed conversion ratios. Statistical comparison of the growth performance and carcass yield grade and quality grade was performed between our three treatment groups using generalized linear model analysis of variance, appropriate for differential expression. Meat quality analyses including color and color stability, tenderness and cooking loss were compared with finishing ration with or without histidine as covariates against measurements of tenderness and cooking loss analyses. Color measurements were analyzed as a repeated measure. Statistical analysis of the data was completed using the Proc. Mixed procedures of SAS software (Statistical Analysis Software version 9.2, Cary, NC, USA). Statistical comparisons were performed between the animals from each of the treatment groups. A mixed statistical model was utilized for analysis. Differences in least squares means (LSM) were compared by the DIFF option. P-values of ≤ 0.05 were considered statistically significant and P-values ≤ 0.10 were considered trends in the data. All data are presented as least square means ± SEM. The Proc. Corr. Procedures of SAS were used to evaluate for pen effect. Additionally, correlations or relationships between all other variables were evaluated, such as; free metabolite content vs. color components, growth traits, sensory attributes, and etcetera.

Results & Discussion

Weaned steers (N=48) were implanted using an industry relevant but aggressive implant; Revalor©-XS (TBA/E2) in an attempt to increase their rates of lean growth. Steers were individually fed a total mixed ration (TMR) in Calan gates so that individual intakes for

each treatment animal could be determined and feed was provided to each steer twice daily to allow *ad libitum* access. RP-HIS was top-dressed on the morning feed according to treatment (dose). Three treatments were used, control (no HIS), low HIS (50 g/hd/d), and high HIS (100 g/hd/d). These doses reflect the daily administration of rumen protected-histidine (RP HIS; Balchem Corp.) of which it is reported to be between 55-60% bioavailable histidine.

Growth and feed efficiency

It has been reported that HIS supplementation in sheep with HIS deficient rumen microbes increased the serum levels HIS in four castrated ram lambs and six ewe lambs of similar breeding (Storm & Orskov, 1984). The efficiency of utilization of absorbed AA-nitrogen microbial protein is evaluated by comparing microbial protein absorption from the small intestine and those absorbed through other sources and overall nitrogen retention. Using this approach, it is possible to calculate both the order and extent of AA limitations in microbial protein. Therefore, if HIS is limiting, dietary supplementation should support improved performance even if it is not rumen-protected. This makes it reasonable to hypothesize that dietary HIS supplementation may in fact improve HIS status or serum bioavailability and increase growth and performance traits in growing cattle. After testing that we had no "pen effect," our comparisons of feed intake (DMI; kg/d), individual growth (ADG) and feed conversion ratio (FCR) were assessed. No differences ($P > 0.10$) were detected in total DMI (Figure 2.1), although, some daily treatment differences ($P \leq 0.05$) and trends ($P \leq 0.10$) were detected. Those daily differences are not significant, as daily intake varies based upon many environmental and biological parameters (Fox, 1986). The daily DMI over the entire feeding period was not significantly different ($P = 0.22$). We realize that our animals' intake is low compared to the average intake of growing cattle in the same phase of development, but it does

numerically appear that the intakes for the low HIS animals are consistently lower than control and high HIS during the first phase of supplementation. With that in mind, it is possible that there is a small decrease in intake that is limited in terms of significance when fed using the Calan gate system. Interestingly, the low HIS group did not show any reduction in growth in the early phase (days 1-21) of supplementation. This may seem trivial but it does indicate that our level of HIS supplementation did not adversely affect growth or intake as has been reported in other studies. No difference in starting live weight, end live weight (Table 2.1) or overall treatment live weight was detected in steers receiving HIS at either dose, although, according to Kasaoka et al. (2004), histidine supplementation (allowed free access to diets containing 0% histidine, 1.0% histidine, 2.5% histidine, or 5% histidine for 8 d) suppressed food intake and fat accumulation in a lab strain of rats, although comparing a monogastric rodent to a large ruminant is difficult by way of differences between feed utilization.

A study by the same group indicated a negative correlation between energy intake and histidine per protein intake, suggesting that histidine supplementation suppresses appetite by activating histamine neurons (2002). Again we report no difference ($P > 0.10$) in our F:G ratio in HIS supplemented steers. While it may be true that our level of HIS supplementation did not impact intake, we are aware that the use of the Calan gate system inherently decreases feed intake by as much as 15% in growing animals, which may have been the predominant influence on intakes. In addition, during the final two weeks of the feeding period, temperatures and humidity fluctuated frequently and as would be expected this environmental condition decreased feed intake (Figure 2.1).

Histidine has been reported as a limiting AA for growing cattle in prior studies (Chalupa et al., 1973; Greenwood & Titgemeyer, 2000). Our own prior data indicated that

supplementation with 50g/hd/d rumen protected histidine to growing beef steers for 129 d pre-harvest trended towards increased ADG (Thornton et al., 2015). Furthermore, in a study by Ma et al. (2010), finishing pigs were supplemented with carnosine, a histidine metabolite for eight weeks. While they observed no difference in growth traits, they noted that the CARN utilization between animals was variable. Utilization was assessed by two methods; they directly assessed the CARN levels within the serum and indirectly evaluated antioxidant and enzyme activity within the tissue using PCR. Previous research is limited in terms of inclusion of supplemental HIS for growing cattle. It is important to consider that supplemented dietary carnosine would be exposed to the variable effects of serum carnosinase prior to its ability to be taken up by muscle tissue, whereas supplementation with HIS and assuming dietary alanine is not limiting the muscle cells can utilize these amino acids to synthesize carnosine. To date, much research has focused on effects of HIS in dairy cattle or swine with very little focused on high-performing finishing beef cattle.

As stated, our study employed an aggressive combination growth implant (Revalor-XS®) in order to stimulate maximal growth, and partially compensate for the expected decrease in performance when fed indoors using the Calan gate system. We report that rumen-protected HIS supplementation trended towards an increase in ADG ($P = 0.07$) for the high HIS group during days 1-21 (1.62 kg/d ± 0.44), 42-60 (1.73 kg/d ± 0.46), and the final 50 days (1.54 kg/d ± 0.32) of feeding. An additional trend for increase in ADG (1.59 kg/d ± 0.46) ($P = 0.07$) was observed in the low dose steers through days 42-60 (Table 2). No difference was observed in FCR in our cattle ($P > 0.10$) (control: 3.13; low HIS: 3.25; high HIS: 3.18 ± 0.34 kg feed/kg gain) (Table 2.1). Note that we observed decreased intake and limited growth performance in these cattle, thus a greater effect of HIS supplementation on FCR may be observable in different

feeding scenarios. No difference (P = 0.39) was observed in the live weights across treatment groups during the feeding period. Although the DMI may have been slightly lower in the first segment of feeding, it does not appear to have affected the live weight of the animals. Again, while we hypothesized that HIS may be limiting in our finishing cattle and that this may be evident by enhanced growth in our HIS supplemented steers it would be premature and possibly a type two statistical error to assume that histidine is not limiting in finishing cattle based on our current measurements alone.

Additionally, our rations were not quantified based on AA profile, although they were theoretically balanced in terms of lysine and methionine content based on ration formulation software and feed commodities used. It should also be noted that the ration included a rather high proportion of corn, which is known to be higher in HIS content than other commonly used feed commodities. The dry distillers grains (DDGs) used within the ration also are known to contain high amounts of vitamin E, a known antioxidant. In lieu of those attributes of the implemented ration, all animals were fed the same and treatment differences were still detected in varying traits.

Our studies assessment of cattle growth and performance traits is limited by our small sample size, use of Calan gates and the decrease in feed intake associated with the abnormally hot and humid weather and extreme temperature fluctuations that were experienced during the final 14 days of our trial. In fact, it is possible that the combination of weather, humidity, and Calan gate individual feeding has a greater effect on DMI than has been previously considered. If this notion is true, then the improvement in growth rate and feed conversion in a bunk fed scenario, under more temperate weather conditions, may be greater than that seen in the present study. In addition, the genetics of the animals used may have had an effect. It is worth

mentioning that no treatment specific detriments were observed in any growth or performance traits in the HIS supplemented groups, which should always be assessed and reported in feeding trials. Further studies with a larger sample group and a genetically diverse population are needed to more effectively evaluate the optimal level of HIS administration, feeding period, and efficacy of rumen-protected HIS for improving average daily gain and FCR.

Carcass cutability and quality

No differences ($P > 0.10$) (Table 2.3) were observed in hot carcass weight (control: 415 kg ± 5.51 , low HIS: 407 kg ± 5.51, high HIS: 416 kg ± 5.51), 12^{th} rib back fat (control: 0.65 in ± 0.05, low HIS: 0.57 in ± 0.05, high HIS: 0.58 in ± 0.05), rib eye area (control: 15 in^2 ± 0.42, low HIS: 14.7 in^2 ± 0.42, high HIS: 15.4 in^2 ± 0.42), final yield grade (control: 3.31 ± 0.21, low HIS: 3.12 ± 0.21, high HIS: 3.00 ± 0.21), or degree of marbling (control: 461 ± 28.65, low HIS: 446 ± 28.65, high HIS: 473 ± 28.65). To date the authors are aware of very limited previous research published on the effects of rumen-protected HIS on finishing steers' carcass traits. This study provided no observable or measurable carcass trait differences when supplemented with 50 g/hd/d or 100 g/hd/d in comparison to the non-treated steers. Although HCW did not increase, no decrease was observed indicating that the supplementation of steers with RP HIS does not limit growth or dressing percentage.

Product quality

According to consumer surveys, tenderness is the most important palatability trait of meat products (Morgan et al., 1991; Koohmaraie et al., 1995; Huffman et al., 1996). Warner-Bratzler Shear Force (WBSF) is the most commonly used method of measuring tenderness in beef products (Derington et al., 2011). There was no difference ($P > 0.10$) in shear force

between HIS treatments and control, indicating that although HIS supplementation did not increase tenderness, no detrimental effects in tenderness were observed (Table 2.4).

Post-mortem muscle changes are accompanied by increased oxidation of myofibrillar proteins resulting in the conversion of amino acids such as histidine to carbonyl derivatives (Levine et al. 1997). These carbonyl derivatives have the ability to decrease the functionality of proteins resulting in water-loss and purge of the product (Xiong & Decker, 1995). Potentially in contradiction to our hypothesis and expectation that HIS supplementation would increase antioxidant content and accordingly decrease protein denaturation, no differences ($P > 0.10$) were observed in cooking loss, cooking time, or retail purge/drip-loss in either muscle analyzed. This suggests that antioxidant levels are not the primary determining variable of protein denaturation or WHC, but rather are due to a number of other factors such as pH. This data indicates the fact that our HIS supplemented groups showed no significant increase in anserine or carnosine levels.

It is well supported that beef product pH is positively correlated to microbial growth (Rhee et al., 1997), although it is negatively correlated to L*, a*, and b* scores (Page et al., 2001). In addition, product pH can indicate product WHC, flavor, cooking loss, protein denaturation, and many other meat quality attributes. As stated, the metabolites anserine and carnosine protect proteins from oxidation and glycation as well as provide pH buffering within the muscle (Kubomura et al., 2009). Muscle pH was measured immediately before cooking for taste panel analysis. No difference was observed in the post-mortem pH of the GM (control: 5.61, low HIS: 5.60, high HIS: 5.61, ± 0.02). Counterintuitively, a trend ($P = 0.09$) for lower pH values in the high HIS group in comparison to the control group was observed in the LL steaks (control: 5.67, low HIS: 5.65, high HIS: 5.63, ± 0.02) (Table 2.4). Although this

measurement of pH is only based on ultimate pH and not rate of pH decline, it can be predicted that this reduction in final pH can be due to longer metabolic activity generating increased proportions of metabolic acids or other scenarios such as activation of calpains which degrade myofibrillar proteins allowing water loss. To the authors' knowledge, limited previous research has been published on the effects of rumen-protected HIS on finishing steers' product quality.

Lipid Oxidation

Lipid oxidation is a major cause of decreased meat quality in terms of flavor, color, and shelf-life (Gray & Pearson, 1987), especially given that the products of fatty acid oxidation are associated with rancid or off-flavors (Gray & Pearson, 1994). Oxidation of structural myofibrillar proteins increases water loss and purge causing decreased WHC and juiciness (Crush et al., 1970; Boldyrev et al., 1988; Lonergan et al., 2001; Ma et al., 2010). No published research has evaluated histidine supplementation in beef cattle and its effects on sensory attributes of beef to date. TBARS analysis on d 0 and 9 of simulated retail display revealed no difference ($P > 0.10$) in oxidation values in the GM (D0: control: 0.28, low HIS: 0.28, high HIS: 0.25, ± 0.07 mg MDA/kg; D9 control: 0.61, low HIS: 0.70, high HIS: 0.64, ± 0.04 mg MDA/kg) or LL (D0: control: 0.17, low HIS: 0.19, high HIS: 0.19, ± 0.08 mg MDA/kg; D9 control: 0.44, low HIS: 0.54, high HIS: 0.56, ± 0.08 mg MDA/kg) (Table 2.4). These results show that pre-harvest HIS supplementation has no effect on product quality via oxidative capacity, but there may still be room for detectable improvement of shelf life through reduction of oxidation via the antioxidative properties of ANS and CARN. Just as ANS and CARN have been shown to possess antioxidative properties which can reduce discoloration of the lean surface, these metabolites may also reduce oxidation of lipid and myofibrillar proteins. The antioxidative effects of ANS and CARN will be discussed in further detail in the forthcoming

HPLC section. Further investigation is needed to evaluate the potential for improved shelf-life and reduced oxidation.

Objective Color

Next to price, the appearance and color of beef is the largest deciding factor as to whether or not a consumer decides to purchase the product (Dunsing et al., 1959; Jeremiah et al., 1972; Kropf et al., 1980; Gatellier et al., 2005; AMSA 2012). Increased color stability means increased probability of a consumer purchasing that product, making it more marketable (Wheeler et al., 1996). Color and color stability were measured in the GM and LL using a Hunter MiniScan EZ to obtain L* (lightness), a* (red/green), and b* (yellow/blue) values to calculate hue angle. The muscles were aged for 14 d post-mortem and then cut into 2.54 cm-thick steaks and packaged in Styrofoam trays and oxygen permeable overwrap. Steaks were then placed into a glass fronted retail display case at 3˚C and rotated daily to prevent location effects due to light intensity. Measurements were obtained in duplicate on days 0, 1, 3, 5, 7 and 9 of simulated retail display using a Hunter MiniScan EZ overtop of the overwrapping.

Hue angle is the arctangent (b*/a*) and is a better indicator than any one measurement alone of overall perceived color of the product. It encompasses the yellow and redness factors that are responsible for the bright cherry red color of lean that consumers characterize as most appealing, and lower values are more desirable and indicate more vivid color profiles. Supplementation with RP HIS during the final 60 days of finishing yielded decreased hue angle values for the low HIS product compared to control ($P = 0.005$) as well as high HIS ($P = 0.04$) (Figure 2.3a) treatment groups of the GM indicating more vivid color. Interestingly, increased hue angle values were observed on day 9 of retail display in both the low HIS ($P = 0.006$) and high HIS ($P = 0.003$) treatment groups compared with control LL (Figure 2.3b). Decreases in

hue angle indicate overall improvements in color stability of beef products (Little, 1975, Wheeler et al., 1996). The confounding results between the two tested muscles lend to the increased complexity of color and color stability of lean muscle, as well as muscle to muscle variation. As these two muscles are classified as having markedly different color stabilities, it is reasonable to think that the quantity or activity of metmyoglobin reductase may be influenced by the presence or absence of HIS, ANS, or CARN, yielding a variation in color component effects. Again, these measurements were taken prior to our actual quantification of our HIS, ANS and CARN by HPLC.

Supplementation also increased the L* values for the low HIS GM product on days 3 ($P = 0.03$) (control: 43.53; low HIS: 45.96; high HIS: 43.56 ± 1.08) and 7 ($P = 0.003$) (control: 41.40; low HIS: 44.65; high HIS: 42.41 ± 1.08) of retail display in comparison to the control steaks and high HIS steaks (Figure 2.4a). In addition, a treatment effect was observed for the low HIS GM product throughout the retail display period ($P = 0.05$), indicating that the low dose treatment was significantly brighter than the control group on days 3 and 7 of retail display (Table 2.5). These improvements in brightness are significant and have the potential to impact the product to an extent that might be detected visually, allowing for the potential for increased selection of product by consumers. Interestingly, no difference was observed in L* for the LL steaks (Figure 2.4b).

An interaction between days and treatment ($P = 0.04$) was observed in the GM product for a* (red/green), yielding redder lean color for the low HIS product. Additionally, a trend for lower a* values were observed for the low HIS group over high HIS and control on day 3 of retail display ($P = 0.08$) (control: 27.74; low HIS: 26.19; high HIS: 27.71 ± 0.78) as well as higher a* values on day 9 ($P = 0.07$) (control: 13.83; low HIS: 15.50; high HIS: 14.16 ± 0.78)

(Figure 2.5a). The high HIS LL steaks had greater a* values than control on day 0 of retail display, indicating redder lean pigment (P = 0.05) (control: 24.25; low HIS: 24.75; high HIS: 25.51 ± 0.63) (Figure 2.5b). Although a* values decrease more drastically within the GM product, both the GM and LL seem to gradually decrease in a* values from days 1-9 of retail display, which agrees with published literature on the subject. Additionally, it appears that while not a significant difference, the a* value for the HIS treated LL steaks tends to decrease numerically on day 9 in comparison to control. A trend was observed for lower b* value in the low HIS GM steaks on day 3 (control: 25.61; low HIS: 24.32; high HIS: 25.73 ± 0.75) of retail display indicating a higher degree of blue pigment (P = 0.06) (Figure 2.6a). The b* value (Figure 2.6b) tended to be lower for the low HIS LL treatment on day 5 of retail display (P = 0.06), indicating a tendency for an increase in blue pigment (control: 30.71; low HIS: 29.85; high HIS: 31.44 ± 0.84) (Table 2.5). In both muscles, a steady decline in b* value is observed with little apparent influence from the HIS supplementation.

Subjective Color

Subjective color measurement was conducted via trained lab personnel following the AMSA meat evaluation guide (AMSA, 2012) on days 0, 1, 3, 5, 7, and 9 of simulated retail display. Steaks were evaluated on an 8-point scale, with higher values meaning darker and lower values meaning lighter and brighter. The low HIS group of GM steaks had significantly more oxygenated lean color (youthful bright cherry red) than control and the high HIS group on days 5 (P = 0.009) and 9 (P = 0.004) of retail display (Figure 2.7a). The high HIS GM product was brighter cherry red than the control product on day 5 of retail display (P = 0.05). In addition, the high HIS LL product proved to have brighter cherry red lean color values on day 9 (P = 0.01) of retail display compared to control (Table 2.6) (Figure 2.7b). All of these

improvements in oxygenated lean color are detectable by the human eye and thus provide a visual effect and could allow this product to become more appealing to consumers.

In the GM steaks, a treatment effect ($P = 0.002$) as well as an interaction between treatment and days ($P < 0.0001$) was observed for decreased browning on the surface of the product. On days 7 ($P = 0.0002$) and 9 ($P < 0.0001$), low HIS treatment had significantly less surface browning than control in the GM steaks. The same was observed for the high HIS group in comparison to control (d 5: $P = 0.0002$; d 9: $P < 0.0001$) (Figure 2.8a). The low and high HIS LL steaks had significantly less browning on days 5 ($P = 0.02$), 7 ($P <0.0001$) and 9 ($P = 0.001$) of retail display in comparison to control (Figure 2.8b) (Table 2.6). It appears that from day 3 to day 9, both treatment groups improve in terms of browning numerically. This discovery indicates that supplemental HIS significantly decreased the visual browning of the product, thus allowing for it to potentially remain marketable for longer.

Both the low HIS and high HIS steaks were significantly less discolored than the control on day 9 ($P < 0.0001$) of retail display in the GM product. Additionally, the high HIS GM steaks tended to be less discolored than the low HIS group on day 7 ($P = 0.06$) (Figure 2.9a). The high HIS steaks from the LL group tended to be less discolored than control and low HIS on day 7 ($P = 0.10$) and were found to be significantly less discolored than control on day 9 ($P = 0.04$) of retail display (Table 2.6) (Figure 2.9b). A steady rate of discoloration increase is observed, with a steeper rate within the control product. In both muscles, a trend was observed by day 7 for improved discoloration in the HIS product that was considered significant by day 9 of display. It is known that the rate of discoloration would be faster in product aged longer prior to retail display (Colle et al., 2015).

A low HIS treatment effect (P = 0.01) as well as a day by treatment interaction (P = 0.0008) was observed for percent surface discoloration (evaluated as a visual % of the surface discolored) in the GM product. On day 7, the high HIS GM steaks tended to have less % surface discoloration than the low HIS and control (P = 0.06) and both treatments proved to have less discoloration than control on day 9 (P < 0.0001) (Figure 2.10a). A decrease in surface discoloration was observed in the high HIS LL product over the control (P = 0.03) on day 9 of retail display (Table 2.6) (Figure 2.10b). The same general trend is noted for surface discoloration as the muscle stays relatively uniform until day 5 and then begins to drastically discolor through to day 9. Although our data indicates significant improvements by day 9, this may not be impactful as it is outside of the normal time a given product remains in a retail display case within the US industry. A day by treatment interaction (P = 0.03) was observed for color uniformity in the GM product throughout retail display. On day 9, the high and low HIS GM steaks were more uniform than control (P = 0.001) (Figure 2.11a). On days 7 (P = 0.06) and 9 (P= 0.06), the high HIS LL steaks tended to be more uniform than control (Table 2.6) (Figure 2.11b). Uniformity scores remained relatively stable until days 7 and 9 of display, when they then increased numerically, indicating less uniformity. It is also observed that on those days, dose of HIS may have played a part in improving uniformity of the product.

Overall, these data indicate that animals supplemented with HIS are less prone to discoloration and browning during retail display, and even improved in terms of lean pigment brightness, redness, yellowness, and uniformity of the lean surface. We tend to focus on a* with beef, and the trend for an improvement in a* in GM at day 9 due to treatment is worth noting. Steaks from control animals had a lower a*, which indicates a less red color. Based on these findings, the GM and LL from animals supplemented with HIS would be more color stable

during retail display than those of untreated controls receiving no HIS supplement, potentially enhancing value and marketability of the product, especially in the GM since it is considered moderately color unstable. During retail display, many products are discolored due to oxidation, light exposure, and varied oxygen concentrations. Therefore, this may translate into an economic benefit for the retailer. Oxidation of lipid and protein cause discoloration and disruption of the color producing agents within meat (Ma et al., 2010). Several antioxidants, namely vitamins A and E, as well as carnosine (histidine-containing dipeptide), have been shown to improve the color stability of beef (Sanches-Escalante et al., 2001).

HIS, and its metabolites, ANS and CARN have been shown to have antioxidant properties (Boldyrev et al., 1988, Wade and Tucker 1998), and carnosine has been shown to inhibit lipid oxidation (Decker and Faraji, 1990). Previous research is limited in reporting the effects of pre-harvest RP HIS or its dipeptides on color. Thornton et al. published data showing that steers receiving RP HIS for 129d pre-harvest had increased a* ($P = 0.004$) and b* ($P = 0.05$) color values during the 9 d simulated retail display (Thornton et al., 2015). Evaluation of the top sirloin following AMSA color evaluation guidelines demonstrated that there was less ($P \leq 0.01$) browning, discoloration and surface discoloration in the steaks from steers receiving histidine (Thornton et al., 2015). Moran et al. (2012) showed that treatment of fattening lambs with carnosic acid delayed lipid oxidation and improved color attributes of the *gluteus medius* muscle. Additionally, this same study reported that meat texture was equally improved and decreased cholesterol oxidation in the same product. A study in pigs increased the a* value after supplementation with carnosine for eight weeks prior to slaughter (Ma et al., 2010).

Several studies have treated meat products post-fabrication with carnosine and other antioxidants. Rosemary powder (containing carnosic acid) (1000 ppm) and rosemary with

ascorbic acid (500 ppm) were applied to ground beef patties and inhibited the formation of metmyoglobin as well as lipid oxidation (Sanchez-Escalante et al., 2001). Lee et al. (1990) treated ground beef with 1 percent carnosine and found that browning, metmyoglobin development, and lipid oxidation were reduced. In addition, overall flavor and appearance of the product was improved after carnosine treatment (Lee, 1997; O'Neil et al., 1998). Beef patties treated with carnosine displayed increased a* or redness values during retail display in addition to decreased lipid and myoglobin oxidation. While post-fabrication application of color stabilizing products shows some improvements, there are benefits to elevating natural anserine and carnosine levels through pre-harvest intervention. By nutritional intervention, the risk of introducing potentially health hazardous pathogens through direct application of product to the lean surface is eliminated. In addition, this reduces the labor required by the packer or retailer by the application of a feed additive. Our results indicate that the improvements observed in the color of product through dietary HIS supplementation are of a similar order of magnitude as those reported by authors such as Liu et al. (2014) and Stewart et al. (1969) through dietary vitamin E supplementation. Finally, anserine and carnosine have the potential to improve many other aspects of product quality that are not affected by a simple product application such as rosemary extract.

In the present study, it was shown that supplemental RP HIS during the final 55 d of finishing improved color and color stability of the GM and LL when subjected to 9 d simulated retail display. 50 g/hd/d of RP HIS yielded greater improvements in color than the high HIS 100 g/hd/d group. We hypothesize that this is due to increased antioxidative capacity that reaches a peak effective dose. When concentrations of metabolites reach a certain point, the rate of breakdown and export increases, which then diminishes efficiency of the metabolite

formation. These results varied, but were most apparent within the GM muscle. The ability to reliably improve sensory and product characteristics of beef is an indispensable asset and may boost the value of each carcass. This increase in color stability and consumer acceptability will improve consumer perception, allowing the potential for increased sales and consumption of beef products.

Consumer perception

Lipid oxidation is a major cause of decreased meat quality in terms of flavor, color, shelf-life, etcetera (Gray & Pearson, 1987). Products formed through oxidation of fatty acids are associated with rancid or off-flavors that impact the consumer's eating experience, and thus are undesirable (Gray & Pearson, 1994). In addition, oxidation of membranes and myofibrillar proteins has the ability to decrease the structure of proteins resulting in water-loss and increased purge of the product (Xiong & Decker, 1995) which impacts the perception of tenderness, juiciness and mouth feel.

In this experiment, the product was evaluated by an untrained taste panel to assess tenderness, juiciness, flavor, texture, and the incidence of off-flavors. Demographic data from the panelist set was evaluated for any attributes that may affect data and reported (Table 2.7). Each panel was carried out in a single day and consisted of 72 untrained panelists. Steaks were cooked to an internal temperature of 71˚C on open hearth broilers, and each consumer evaluated four pieces in terms of juiciness, flavor, texture, tenderness, and acceptability on a nine-point scale. Additionally, panelists were asked to indicate the incidence of off-flavors and if they would be willing to purchase the product in the future.

Consumers overall reported that the low HIS steaks tended to be juicier but no different than control (P= 0.07) (control: 5.09, low HIS: 5.42, high HIS: 4.59, ± 0.17) than the control

and high dose steaks in the GM group, while the high HIS steaks were significantly drier or less juicy than the other two treatments (P = 0.003) (Table 2.9). Consumers also found the low HIS GM product more acceptable (P= 0.05) than the high HIS GM product (control: 5.89, low HIS: 6.11, high HIS: 5.64, ± 0.15). Almost 77% of panelists said they would purchase the low dose GM product in comparison to control (61%) and high dose (60%). No differences (P > 0.10) in flavor (control: 5.71, low HIS: 5.92, high HIS: 5.71, ± 0.18) or the incidence of off-flavors were observed in the GM product. Interestingly, no differences were detected in any of the LL steaks among treatments (Table 2.8).

As mentioned, both ANS and CARN are antioxidants that can stabilize chromophores in meat to improve color and stabilize lipids to improve flavor and stabilize proteins to enhance juiciness through improved water holding capacity (WHC). The above data indicate that supplemental HIS during finishing of beef steers increased juiciness of the product, as well as, acceptability and overall satisfaction reported by consumers. Furthermore, our data indicates that high HIS supplementation is detrimental to juiciness of the product, although there was no correlation or relationship of metabolite content to cook time, temperature, purge, cook loss, pH, color, sensory attribute, or any other variable evaluated. In addition, there was no correlation between any live animal pen group, location, weight, intake, or carcass quality. Increased antioxidant content may also reduce the incidence of off-flavors within the product, thus leading to a more consistent eating experience. Although differences were only observed in the GM steaks, further investigation and analysis of additional muscles is necessary to determine efficacy across the entire carcass. It may be important to re-evaluate some of these sensory attributes using a trained taste panel in the future,

Metabolite Content

Histidine (HIS) is considered the third most limiting amino acid (AA) in growing ruminants (Chalupa et al., 1973; Greenwood et al., 2000) following methionine and lysine. In this study, the diets were balanced via software and feed components for lysine and methionine content, but were not chemically analyzed for AA profile. Furthermore, HIS is converted to two metabolites within the muscle; carnosine (CARN) and anserine (ANS). These metabolites provide antioxidant properties that may preserve color, prevent rancidity, prevent oxidation in general, and may be of human health importance. Both of these compounds are found in high concentrations in vertebrate mammalian muscle and brain tissues, in particularly high concentrations within fast-twitch muscle fibers (Bump et al., 1990; Dunnet et al., 1997) which may indicate that more of these antioxidants are formed and stored through muscle fiber hypertrophy. Additionally, anserine and carnosine are neuroprotective in nature and it's possible that if provided in the human diet they may reduce Alzheimer's, dementia, and other neurodegenerative diseases. If these relationships were to be tested and found to hold true, this could allow for the marketing and expansion of a more healthful beef product and increase beef consumption within the US and internationally.

Tissue samples from the GM and LL were thawed, cut into strips, snap frozen, ground under liquid nitrogen, homogenized, protein extracted, and derivatized under vacuum and nitrogen using a PicoTag Workstation (Waters). The derivatized samples were then analyzed using HPLC to evaluate free HIS, ANS, and CARN content. HPLC analysis showed that there was no difference ($P > 0.10$) in the free HIS (control: 0.145, low HIS: 0.185, high HIS: 0.166, ± 0.36 mg/g), ANS (control: 0.213, low HIS: 0.202, high HIS: 0.156, ± 0.04 mg/g), or CARN (control: 0.049, low HIS: 0.042, high HIS: 0.059, ± 0.02 mg/g) contents within the muscle in the GM product. Interestingly, the low HIS LL product contained higher ($P = 0.04$) free HIS

content than both the control and high HIS groups (control: 0.049, low HIS: 0.076, high HIS: 0.051, ± 0.01 mg/g). No difference (P > 0.10) was observed in free ANS (control: 0.149, low HIS: 0.141, high HIS: 0.133, ± 0.02 mg/g) or CARN (control: 0.010, low HIS: 0.010, high HIS: 0.010, ± 0.0009 mg/g). content (Table 2.10) (Figure 2.12a-2.12b). We also observed that the quantity of metabolites within the tissue varied by muscle, with the GM containing higher amounts than the LL. This may be due to the fact that these muscles are made up of different fiber type compositions which in turn, changes the metabolism of the muscle. Our muscles were not sampled immediately post-mortem and remained frozen for a period of time before analysis, so it is possible that oxidation of the tissues occurred affecting our ability to extract proteins or even maintain the stability of the metabolites. There is large individual variability among these groups which may mask any effect that may be present. Additionally, due to being housed inside and in confinement, our animals received an injection of vitamins A, D, and E to prevent deficiency which could have caused an increase in vitamin E stores (known antioxidant) within the tissues. This is confounded by the use of dry distillers grains within our ration, which are known to be a source of natural vitamin E, may have caused our animals to be at an elevated level of vitamin E status, reducing the already substantial effects observed on color through dietary RP HIS.

Dunnet and Harris (1990) found increased carnosine levels in the GM muscle of horses after supplementation with β-alanine and L-hisitidine, however there was no increase in HIS levels. Thornton et al. (2015) saw increased HIS, ANS, and CARN levels within the GM and LT muscles of finishing steers fed a potato-by-product ration but did not see any increase in metabolites with RP HIS supplementation over 129 d pre-harvest. Factors including genetics, diet composition, and the withdrawal of feed, including RP HIS, for over 24 h prior to harvest

may have affected the deposition and retention of the metabolites within the muscle. Additionally, the fluctuation in environmental conditions that decreased the feed intake of the animals, namely temperature and humidity, during the final 14 d of the feeding period may have decreased the deposition of metabolites within the muscle as well. Lastly, breed characteristics may have had an effect as the experimental cattle in the present study were of the same breed composition and origin, versus the cattle used in Thornton's study that were of varying breed types, size, ages, and genetic makeup in addition to cattle sourced from the same herd as the cattle in the present study (2015). Watanabe et al. (2004) reported that histidine, anserine and carnosine levels vary based on breed type and age of beef cattle showing a drastic increase in histidine and anserine, as well as a decrease in carnosine when animals exceeded 25 months of age. In addition, Japanese Black cattle had higher anserine and lower carnosine concentrations than Japanese Shorthorn and Japanese Holstein cattle. As current literature on the effects of RP HIS supplementation is limited, further investigation is required to evaluate the potential elevation of metabolites within the lean tissue as there may still be an effect. A future study should determine if there is an increase in blood HIS due to absorption of the RP-HIS provided within the ration, although sufficiently measuring blood HIS levels would be extremely labor intensive and cost prohibitive. Additionally, anserine and carnosine within the blood are broken down by anserinase and carnosinase, therefore, it would be difficult to measure serum levels of ANS and CARN.

Implications

Per-capita beef consumption within the U.S. is declining, thus proving the importance of the increase of the marketability, consistency, and exportability of U.S. beef. Furthermore, shipping and day from harvest to marketing may rise over the next decade and therefore color

stability may become a quality of increasing importance to ensure that US beef has the most appealing marketable appearance relative to other beef export nations, such as Brazil. Research driven to identify proven and acceptable methods of enhancing carcass growth, yield, meat quality, and/or improved sensory attributes or healthfulness may provide new marketability and support the beef industry. Inevitably, this method of supplementation has the potential to improve beef quality and yield through a simple nutritional pre-harvest intervention, although we saw no effects or detriments. Alteration of the nutrients provided is the easiest manner to enhance or change the product, and potentially increase the value and marketability of said product. Pre-harvest dietary treatments that favorably alter the quality aspects of beef are also the most accepted by the general population of consumers. In finishing cattle diets, very little research has been conducted to determine the effects that supplemental His has on growth traits, carcass characteristics, and end-product quality/consumer acceptability. We have shown that supplementing finishing beef cattle with rumen-protected (RP) His has no detrimental effects on any tested aspect of growth, efficiency, carcass traits, product quality, or metabolite content. Supplemental HIS at the doses tested herein trend to increase growth. In addition, supplemental HIS improves color and color stability (GM and LL steaks) replicated across two separate trials, and some consumer sensory attributes such as juiciness, acceptability, satisfaction, and in the end, overall marketability of the product (in GM steaks). This was the first study to assess the sensory impact of pre-harvest HIS supplementation. It appears that pre-harvest rumen-protected HIS supplementation yields a more consistent and more color stable product.

We have demonstrated that the low dose (50 g/hd/d) RP-HIS supplementation has greater quantifiable benefits than the high dose (100 g/hd/d) in many aspects of product quality and consumer perception as well as, free HIS content, while the high dose shows higher impact

in terms of growth. The potential for an increase in metabolite deposition has the ability to boost the healthful aspects of beef and allow for the possible creation of a “niche” or “custom” beef product. However, we did not demonstrate a numerical increase in quantified antioxidants. In addition, adding a simple feed ingredient that yields these benefits is a practical means for product enhancement, and can be adapted to fit production scenarios at the feedlot. The advantages of which may be relevant given the continual rise in age related dementia and Alzheimer’s in humans. While the metabolite content of this product has produced surprising results, and only increased in one muscle, future research should be directed toward evaluating efficacy of this treatment type across muscles from the whole carcass. Narrowing down an appropriate, effective and optimal dosage, as well as timing of RP HIS is the next important step in determining the utility of these results. Although the RP HIS used in this study is not economically feasible for large scale application, lower cost sources or production of RP-HIS or use of natural sources of histidine, namely canola, could be explored to allow for economically beneficial application of this practice within beef production systems.

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Table 2.1.

Intake, growth and feed conversion traits of animals over final finishing phase

	Treatment			
	Control[1§]	Low HIS[2§]	High HIS[3§]	P-Value
FCR	3.13 ± 0.10	3.25 ± 0.18	3.18 ± 0.17	0.87
Daily DMI (kg)	10.37 ± 0.10	9.85 ± 0.30	10.27 ± 0.21	0.22
Starting LW (kg)	350 ± 6.57	343 ± 7.62	357 ± 9.11	0.38
Harvest LW (kg)	669 ± 7.75	657 ± 7.57	671 ± 9.11	0.42

FCR, DMI, and live weights for steers (N=48) over final 60 d of finishing across treatments. No differences were observed between treatment groups in any variable.

FCR: Feed Converstion Ratio (kg feed : kg gain)

DMI: Dry Matter Intake (kg/d)

LW: Live Weight (kg)

§ Values indicate mean ± SEM

[1] No histidine supplementation, N=16

[2] 50 g/hd/d histidine supplementation, N=16

[3] 100 g/hd/d histidine supplementation, N=16

Table 2.2.

Steer average daily gains (kg) for the final 60 d of finishing across treatment groups

	Treatment			
	Control[1§]	Low HIS[2§]	High HIS[3§]	P-Value
ADG (kg/day)				
Duration (60 d)	1.53[a] ± 0.05	1.43[a] ± 0.06	1.52[a] ± 0.07	0.24
Days 1-21	1.56[a] ± 0.10	1.46[a] ± 0.15	1.62[b] ± 0.17	**0.07**[†]
Days 22-41	1.80[a] ± 0.09	1.62[a] ± 0.13	1.73[a] ± 0.10	0.38
Days 42-60	1.44[a] ± 0.12	1.59[ab] ± 0.10	1.57[b] ± 0.09	**0.07**[†]
Final 50 days	1.23[a] ± 0.11	1.37[a] ± 0.09	1.46[b] ± 0.10	**0.06**[†]

Steer ADG (average daily gain, N = 48) over final 60 d of finishing across treatment groups. A trend was observed for increased growth in the high HIS group on days 1-21, 42-60, and the final 50 days (P= 0.07, 0.07, 0.06 respectively. A trend was also observed for increased growth for the low HIS group on days 42-60 (P = 0.07).

Values "a" and "b" considered statistically different at P<0.10

[†] P-values show tendencies

[§] Values indicate mean ± SEM

[1] No histidine supplementation, N=16

[2] 50 g/hd/d histidine supplementation, N=16

[3] 100 g/hd/d histidine supplementation, N=16

Table 2.3.

Carcass data for finishing steers across treatment groups

	Treatment			
	Control[1§]	Low HIS[2§]	High HIS[3§]	P-Value
HCW	415.00 ± 5.42	407.00 ± 6.06	416.00 ± 5.01	0.45
DP	62.20 ± 0.34	62.00 ± 0.39	62.10 ± 0.36	0.93
12th rib fat	0.65 ± 0.04	0.57 ± 0.04	0.58 ± 0.03	0.32
REA	15.00 ± 0.34	14.70 ± 0.25	15.40 ± 0.29	0.29
YG	3.31 ± 0.15	3.12 ± 0.14	3.00 ± 0.15	0.35
MARB	461.00 ± 18.11	446.00 ± 13.90	473.00 ± 26.65	0.64

Supplemental HIS effects on carcass data of steers across treatments. Data was obtained by trained UI personnel at Washington Beef (Toppenish, WA) 24 h post-mortem.

HCW: Hot carcass weight (kg)

DP: Dressing percentage

REA: Rib eye area

KPH %: Kidney, pelvic, and heart fat as percentage of HCW

YG: yield grade

MARB: Marbling score

§ Values indicate mean ± SEM

[1] No histidine supplementation, N=16

[2] 50 g/hd/d histidine supplementation, N=16

[3] 100 g/hd/d histidine supplementation, N=16

Table 2.4. Product data for *gluteus medius* and *longissimus lumborum* from histidine treated steers

Table 4. Product data

	Treatment							
	Gluteus medius				Longissimus lumborum			
	Control[1§]	Low HIS[2§]	High HIS[3§]	P-Value	Control[1§]	Low HIS[2§]	High HIS[3§]	P-Value
WBSF	3.41[a] ± 0.15	3.60[a] ± 0.15	3.45[a] ± 0.12	0.52	3.01[a] ± 0.10	3.07[a] ± 0.11	3.19[a] ± 0.14	0.54
pH	5.61[a] ± 0.02	5.60[a] ± 0.01	5.61[a] ± 0.01	0.87	5.67[a] ± 0.02	5.65[a] ± 0.01	5.63[b] ± 0.01	0.09[†]
Cook Time	14.30[a] ± 1.08	16.63[a] ± 1.20	17.50[a] ± 1.77	0.25	15.19[a] ± 1.98	16.50[a] ± 1.28	16.50[a] ± 1.08	0.42
Percent Cook Loss	23.80[a] ± 0.01	26.40[a] ± 0.01	26.10[a] ± 0.01	0.24	22.7[a] ± 0.02	24.3[a] ± 0.01	26.5[a] ± 0.01	0.14
Percent Retail Loss	2.45[a] ± 0.002	1.92[a] ± 0.002	2.18[a] ± 0.002	0.29	1.12[a] ± 0.001	1.27[a] ± 0.001	1.21[a] ± 0.001	0.53
Lipid Oxidation d0	0.28[a] ± 0.02	0.28[a] ± 0.03	0.25[a] ± 0.02	0.59	0.17[a] ± 0.03	0.19[a] ± 0.03	0.19[a] ± 0.01	0.80
Lipid Oxidation d9	0.61[a] ± 0.07	0.70[a] ± 0.11	0.64[a] ± 0.06	0.91	0.44[a] ± 0.05	0.54[a] ± 0.09	0.56[a] ± 0.07	0.12

Effects of HIS supplementation on product quality from finishing steers (N=48) across treatment groups. A trend for decreased pH value was observed in the high HIS group (P = 0.09). No other differences were observed.

Values "a" and "b" considered statistically different at P<0.10

Cook Time: minutes to 71˚C

WBSF: Warner-Bratzler Shear Force (kg)

Lipid Oxidation: values reported as mg MDA/kg meat

* P-values are significant P ≤ 0.05

[†] P-values show tendencies

[§] Values indicate mean ± SEM

[1] No histidine supplementation, N=16

[2] 50 g/hd/d histidine supplementation, N=16

[3] 100 g/hd/d histidine supplementation, N=16

Table 2.5. Instrumental color for *gluteus medius* and *longissimus lumborum*

	Day of Display	Treatment			
		Control[1§]	Low HIS[2§]	High HIS[3§]	P-Value
Gluteus medius					
Lightness	0	40.31^{a} ± 0.58	40.91^{a} ± 0.63	40.97^{a} ± 0.75	0.54
	1	42.34^{a} ± 0.52	42.87^{a} ± 0.72	42.66^{a} ± 0.83	0.62
	3	43.53^{a} ± 0.54	45.96^{b} ± 0.93	43.56^{a} ± 0.84	**0.03***
	5	41.56^{a} ± 0.73	43.32^{a} ± 0.77	43.21^{a} ± 0.94	0.11
	7	41.40^{a} ± 0.71	44.65^{b} ± 0.82	42.41^{a} ± 0.81	**0.003***
	9	40.08^{a} ± 0.79	41.21^{a} ± 0.87	40.86^{a} ± 0.87	0.47
Yellow/Blue	0	26.57^{a} ± 0.76	26.86^{a} ± 0.33	27.45^{a} ± 0.55	0.43
	1	30.80^{a} ± 0.50	31.02^{a} ± 0.56	30.05^{a} ± 0.49	0.32
	3	25.61^{a} ± 0.46	24.32^{b} ± 0.61	25.73^{a} ± 0.45	0.06†
	5	23.82^{a} ± 0.48	23.33^{a} ± 0.61	23.36^{a} ± 0.41	0.54
	7	21.25^{a} ± 0.48	20.98^{a} ± 0.74	20.85^{a} ± 0.62	0.72
	9	22.00^{a} ± 0.42	22.14^{a} ± 0.45	22.02^{a} ± 0.42	0.86
Red/Green	0	28.32^{a} ± 0.56	28.46^{a} ± 0.65	29.06^{a} ± 0.41	0.54
	1	34.33^{a} ± 0.57	34.62^{a} ± 0.52	33.62^{a} ± 0.44	0.42
	3	27.74^{a} ± 0.56	26.19^{b} ± 0.70	27.71^{a} ± 0.54	0.08†
	5	24.52^{a} ± 0.68	24.12^{a} ± 0.78	24.02^{a} ± 0.59	0.46
	7	19.88^{a} ± 1.09	19.60^{a} ± 1.10	19.70^{a} ± 0.84	0.9
	9	13.83^{a} ± 0.66	15.50^{b} ± 0.76	14.16^{a} ± 0.47	0.07†
Longissimus lumborum					
Lightness	0	42.18^{a} ± 0.59	41.64^{a} ± 0.64	43.13^{a} ± 0.89	0.62
	1	43.16^{a} ± 0.68	43.12^{a} ± 0.58	42.82^{a} ± 0.95	0.76
	3	44.02^{a} ± 1.06	43.46^{a} ± 0.76	43.66^{a} ± 0.73	0.61
	5	40.72^{a} ± 1.23	41.97^{a} ± 0.95	41.62^{a} ± 0.88	0.42
	7	42.95^{a} ± 0.92	42.52^{a} ± 0.92	43.15^{a} ± 0.71	0.7
	9	43.25^{a} ± 0.46	43.65^{a} ± 0.79	43.56^{a} ± 0.74	0.78
Yellow/Blue	0	20.21^{a} ± 0.63	21.06^{a} ± 0.42	21.19^{a} ± 0.65	0.31
	1	31.31^{a} ± 0.46	30.98^{a} ± 0.33	31.87^{a} ± 0.58	0.5
	3	27.95^{a} ± 0.76	27.80^{a} ± 0.48	29.18^{a} ± 0.47	0.11
	5	30.71^{ab} ± 0.74	29.85^{a} ± 0.59	31.44^{b} ± 0.58	0.06†
	7	26.66^{a} ± 0.61	25.59^{a} ± 0.59	26.81^{a} ± 0.75	0.2
	9	19.10^{a} ± 0.67	17.90^{a} ± 0.55	18.55^{a} ± 0.64	0.16
Red/Green	0	24.25^{a} ± 0.50	24.76^{ab} ± 0.41	25.51^{b} ± 0.42	**0.05***
	1	36.39^{a} ± 0.45	36.34^{a} ± 0.30	36.86^{a} ± 0.48	0.43
	3	33.30^{a} ± 0.73	33.60^{a} ± 0.37	34.09^{a} ± 0.50	0.53
	5	34.70^{a} ± 0.68	34.13^{a} ± 0.60	35.11^{a} ± 0.66	0.29
	7	31.08^{a} ± 0.66	30.12^{a} ± 0.84	30.74^{a} ± 1.31	0.49
	9	20.92^{a} ± 1.50	17.86^{a} ± 1.43	18.46^{a} ± 1.59	0.15

Instrumental color values from steers supplemented with HIS. Measurements were taken on days 0, 1, 3, 5, 7, and 9 under simulated retail display with Hunter MiniScan EZ in repeated measures.

Values "a-c" considered statistically different at P<0.10

[†] P-values show tendencies

* P-values show statistically significant at P ≤ 0.05

[§] Values indicate mean ± SEM

[1] No histidine supplementation, N=16

[2] 50 g/hd/d histidine supplementation, N=16

[3] 100 g/hd/d histidine supplementation, N=16

Table 2.6a. Visual color for *gluteus medius*

	Day of Display	Treatment			
		Control[1§]	Low HIS[2§]	High HIS[3§]	P-Value
Gluteus medius					
Oxygenated lean color [1]	0	1.94[a] ± 0.17	1.88[a] ± 0.18	1.63[a] ± 0.18	0.33
	1	1.94[a] ± 0.17	1.88[a] ± 0.18	1.63[a] ± 0.18	0.33
	3	1.94[a] ± 0.17	1.88[a] ± 0.18	1.63[a] ± 0.18	0.33
	5	3.00[a] ± 0.18	2.56[b] ± 0.24	2.31[b] ± 0.25	**0.009***
	7	3.56[a] ± 0.13	3.50[a] ± 0.20	3.25[a] ± 0.21	0.34
	9	4.63[a] ± 0.18	3.88[b] ± 0.15	4.38[a] ± 0.18	**0.004***
Amount of browning [2]	0	1.00[a] ± 0.00	1.00[a] ± 0.00	1.00[a] ± 0.00	1.00
	1	1.00[a] ± 0.00	1.11[a] ± 0.03	1.00[a] ± 0.00	0.76
	3	1.94[a] ± 0.19	1.81[a] ± 0.20	1.81[a] ± 0.14	0.56
	5	2.38[a] ± 0.16	2.06[a] ± 0.18	2.13[a] ± 0.21	0.24
	7	3.44[a] ± 0.16	2.81[b] ± 0.27	2.63[c] ± 0.18	**0.0002***
	9	4.94[a] ± 0.21	3.50[b] ± 0.30	3.63[b] ± 0.20	**<0.0001***
Discoloration [3]	0	1.00[a] ± 0.00	1.00[a] ± 0.00	1.00[a] ± 0.00	1.00
	1	1.63[a] ± 0.15	1.75[a] ± 0.19	1.38[a] ± 0.13	0.28
	3	1.94[a] ± 0.19	2.00[b] ± 0.20	1.88[a] ± 0.16	0.79
	5	2.56[a] ± 0.18	2.19[a] ± 0.21	2.19[a] ± 0.19	0.11
	7	3.19[a] ± 0.14	2.81[a] ± 0.26	2.75[b] ± 0.20	**0.06[†]**
	9	4.63[a] ± 0.40	3.50[b] ± 0.20	3.38[b] ± 0.18	**<0.0001***
Surface discoloration [4]	0	1.00[a] ± 0.00	1.00[a] ± 0.00	1.00[a] ± 0.00	1.00
	1	1.75[a] ± 0.19	1.75[a] ± 0.19	1.50[a] ± 0.16	0.31
	3	2.00[a] ± 0.20	2.00[a] ± 0.44	1.88[a] ± 0.15	0.61
	5	2.81[a] ± 0.21	2.25[b] ± 0.39	2.19[b] ± 0.19	**0.01***
	7	3.65[a] ± 0.16	3.06[b] ± 0.33	3.13[b] ± 0.20	**0.04***
	9	5.25[a] ± 0.21	3.94[b] ± 0.19	4.00[b] ± 0.16	**<0.0001***
Color uniformity [5]	0	2.06[a] ± 0.19	2.38[a] ± 0.20	2.06[a] ± 0.14	0.18
	1	2.06[a] ± 0.19	2.38[a] ± 0.20	2.06[a] ± 0.14	0.18
	3	2.06[a] ± 0.20	2.38[a] ± 0.18	2.19[a] ± 0.14	0.59
	5	2.38[a] ± 0.15	2.44[a] ± 0.19	2.44[a] ± 0.16	0.79
	7	2.69[a] ± 0.12	2.69[a] ± 0.18	2.31[a] ± 0.12	0.11
	9	3.69[a] ± 0.12	3.25[b] ± 0.17	2.94[b] ± 0.14	**0.001***

Visual color values from steers (N=48) supplemented with RP HIS during finishing. Steaks were scored on days 0, 1, 3, 5, 7, and 9 of retail display.

[1] 1 = extremely brightcherry-red, 2 = brightcherry-red, 3 = moderatelybright cherry-red, 4 = slightlybrightcherry-red, 5 =

[2] 1=noevidenceofbrowning,2=dull,3=grayish,4=brownish-gray,5=brown, 6 = darkbrown.

[3] 1 = none, 2 = slight, 3 = small,4 = moderate,5 = extreme.

[4] 1=none(0%),2=slight(1–20%),3 =small(21–40%),4 =modest(41–60%),5 = moderate (61–80%), 6 = extensive(81–100%).

[5] 1=uniform,2=slighttwo-toning,3=smallamountoftwo-toning,4=moderate two-toning,5 = extreme two-toning.

Values "a-c" considered statistically different at P<0.10

[†] P-values show tendencies

* P-values indicate statistically significant at P ≤ 0.05

§ Values indicate mean ± SEM

[1] No histidine supplementation, N=16

[2] 50 g/hd/d histidine supplementation, N=16

[3] 100 g/hd/d histidine supplementation, N=16

Table 2.6b. Visual color for *longissimus lumborum*

		Treatment			
	Day of Display	Control[1§]	Low HIS[2§]	High HIS[3§]	P-Value
Longissimus lumborum					
Oxygenated lean color [1]	0	$1.19^{a} \pm 0.10$	$1.31^{a} \pm 0.12$	$1.19^{a} \pm 0.10$	0.58
	1	$1.19^{a} \pm 0.10$	$1.31^{a} \pm 0.12$	$1.19^{a} \pm 0.10$	0.58
	3	$1.56^{a} \pm 0.13$	$1.50^{a} \pm 0.13$	$1.38^{a} \pm 0.13$	0.41
	5	$1.81^{a} \pm 0.16$	$1.75^{a} \pm 0.19$	$1.50^{a} \pm 0.18$	0.27
	7	$2.38^{a} \pm 0.18$	$2.38^{a} \pm 0.24$	$2.25^{a} \pm 0.28$	0.58
	9	$4.31^{a} \pm 0.20$	$4.06^{ab} \pm 0.17$	$3.75^{b} \pm 0.17$	**0.01***
Amount of browning [2]	0	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	1
	1	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	1
	3	$1.44^{a} \pm 0.13$	$1.25^{a} \pm 0.14$	$1.19^{a} \pm 0.14$	0.37
	5	$1.75^{a} \pm 0.14$	$1.44^{ab} \pm 0.20$	$1.25^{b} \pm 0.19$	**0.02***
	7	$2.81^{a} \pm 0.23$	$2.31^{b} \pm 0.27$	$1.88^{c} \pm 0.29$	**<0.0001***
	9	$4.00^{a} \pm 0.13$	$3.38^{b} \pm 0.13$	$3.31^{b} \pm 0.12$	**0.001***
Discoloration [3]	0	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	1
	1	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	1
	3	$1.06^{a} \pm 0.06$	$1.06^{a} \pm 0.06$	$1.06^{a} \pm 0.06$	1
	5	$1.06^{a} \pm 0.06$	$1.06^{a} \pm 0.06$	$1.06^{a} \pm 0.06$	1
	7	$1.75^{a} \pm 0.17$	$1.75^{a} \pm 0.23$	$1.44^{b} \pm 0.20$	0.10†
	9	$4.06^{a} \pm 0.23$	$3.88^{ab} \pm 0.24$	$3.69^{b} \pm 0.20$	**0.04***
Surface discoloration [4]	0	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	1
	1	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	1
	3	$1.06^{a} \pm 0.06$	$1.06^{a} \pm 0.06$	$1.06^{a} \pm 0.06$	1
	5	$1.06^{a} \pm 0.06$	$1.06^{a} \pm 0.06$	$1.06^{a} \pm 0.06$	1
	7	$1.75^{a} \pm 0.17$	$1.69^{a} \pm 0.20$	$1.44^{a} \pm 0.20$	0.28
	9	$4.56^{a} \pm 0.32$	$4.19^{ab} \pm 0.32$	$4.06^{b} \pm 0.27$	**0.03***
Color uniformity [5]	0	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	1
	1	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	1
	3	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	1
	5	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	1
	7	$1.69^{a} \pm 0.15$	$1.56^{ab} \pm 0.16$	$1.38^{b} \pm 0.15$	0.06†
	9	$3.75^{a} \pm 0.23$	$3.63^{ab} \pm 0.22$	$3.44^{b} \pm 0.20$	0.06†

Visual color values from steers (N=48) supplemented with RP HIS during finishing. Steaks were scored on days 0, 1, 3, 5, 7, and 9 of retail display.

[1] 1 = extremely brightcherry-red, 2 = brightcherry-red, 3 = moderatelybright cherry-red, 4 = slightlybrightcherry-red, 5 = slightlydarkcherry-red, 6 =

[2] 1=noevidenceofbrowning,2=dull,3=grayish,4=brownish-gray,5=brown, 6 = darkbrown.

[3] 1 = none, 2 = slight, 3 = small,4 = moderate,5 = extreme.

[4] 1=none(0%),2=slight(1–20%),3 =small(21–40%),4 =modest(41–60%),5 = moderate (61–80%), 6 = extensive(81–100%).

[5] 1=uniform,2=slighttwo-toning,3=smallamountoftwo-toning,4=moderate two-toning,5 = extreme two-toning.

Values "a-c" considered statistically different at P<0.10

† P-values show tendencies

* P-values indicate statistically significant at $P \leq 0.05$

§ Values indicate mean ± SEM

[1] No histidine supplementation, N=16

[2] 50 g/hd/d histidine supplementation, N=16

[3] 100 g/hd/d histidine supplementation, N=16

Table 2.7. Demographics of consumer panelists

	Longissimus lumborum		***Gluteus medius***	
	n	**%**	***n***	**%**
Age				
18-19	6	8.3	4	5.6
20-29	51	71.8	43	59.7
30-39	7	9.9	8	11.1
40-49	4	5.6	5	6.9
50+	3	4.2	10	13.9
Gender				
Male	41	57.7	25	34.7
Female	30	42.3	46	63.9
Beef meals/wk[1]				
0-1	12	16.9	9	12.5
2-4	44	62.0	47	65.3
5-7	10	14.1	13	18.1
8+	6	8.5	1	1.4
Most consumed[2]				
Ground	40	56.3	47	65.3
Roast	4	5.6	7	9.7
Steak	32	45.1	17	23.6
Other	1	1.4	1	1.4

[1]Please indicate the number of meals a week in which you consume beef: 0-1, 2-4, 5-7, or 8+

[2]Please indicate the form in which you most commonly consume beef: ground, roast, steak, or other

Table 2.8. Consumer panel preferences for the *longissimus lumborum* and *gluteus medius*

	Treatment					
	Longissimus lumborum			*Gluteus medius*		
	Control[1§]	Low HIS[2§]	High HIS[3§]	Control[1§]	Low HIS[2§]	High HIS[3§]
Like most[1]						
Flavor	31.3	28.1	29.5	35.4	38.5	46.3
Tenderness	30.2	34.4	24.2	31.3	21.9	23.2
Juiciness	18.8	20.8	14.7	17.7	17.7	11.6
Texture	13.5	10.4	20	9.4	12.5	7.4
Like least[2]						
Flavor	28.1	25	27.4	26	20.8	15.8
Tenderness	10.4	14.6	16.8	21.9	30.2	26.3
Juiciness	30.2	37.5	25.3	33.3	21.9	37.9
Texture	19.8	12.5	12.6	7.3	12.5	15.8
Off flavor[3]						
Yes	16.7	18.9	16	18.8	12.6	21.3
No	83.3	81.1	83.2	81.3	87.4	78.7
Purchase[4]						
Yes	77.1	71.9	74.5	61.1	76.6	60.6
No	22.9	28.1	25.5	38.9	23.4	39.4

[1]Percentage of panelists that liked that attribute the most

[2]Percentage of panelists that liked that attribute the least

[3]Percentage of panelists that did or did not detect and off-flavor

[4]Percentage of panelists willing or not willing to purchase the product

Taste panel responses (Panelists N = 72 per panel) for LL and GM. Scored using a 9-point scale (9 = like extremely, extremely tender, extremely juicy, and like flavor extremely, respectively; 1 = dislike extremely, not at all tender, extremely dry, and dislike flavor extremely, respectively).

Table 2.9. Consumer Panel Analysis[1] of the *gluteus medius* and *longissimus lumborum*

	Treatment			
	Control[1§]	Low HIS[2§]	High HIS[3§]	P-Value
Gluteus medius				
Acceptability	5.89[ab] ± 0.12	6.11[b] ± 0.11	5.64[a] ± 0.15	**0.05***
Tenderness	5.72[a] ± 0.16	5.78[a] ± 0.17	5.38[a] ± 0.15	0.25
Juiciness	5.09[a] ± 0.18	5.43[b] ± 0.18	4.59[c] ± 0.15	**0.003***
Flavor	5.71[a] ± 0.15	5.92[a] ± 0.16	5.71[a] ± 0.18	0.65
Longissimus Lumborum				
Acceptability	6.63[a] ± 0.13	6.27[a] ± 0.16	6.25[a] ± 0.12	0.79
Tenderness	6.15[a] ± 0.20	6.18[a] ± 0.17	6.17[a] ± 0.16	0.99
Juiciness	5.37[a] ± 0.17	5.43[a] ± 0.20	5.46[a] ± 0.16	0.97
Flavor	5.90[a] ± 0.17	5.72[a] ± 0.18	5.72[a] ± 0.18	0.68

Panelist (N= 72) responses to product from steers (N= 48)treated with RP HIS during finishing.

[abc]Within a row values differ($P < 0.10$)

[1] Scale, 9 = like extremely, extremely tender, extremely juicy, and like flavor extremely, respectively; 1 = dislike extremely, not at all tender, extremely dry, and dislike flavor extremely,respectively.

* P-values show statisically different at $P \leq 0.05$

[§] Values indicate mean ± SEM

[1] No histidine supplementation, N=16

[2] 50 g/hd/d histidine supplementation, N=16

[3] 100 g/hd/d histidine supplementation, N=16

Table 2.10. Free histidine, anserine and carnosine content within *gluteus medius* and *longissimus lumborum*.

	Treatment			
	Control[1§]	Low HIS[2§]	High HIS[3§]	P-Value
Gluteus Medius				
Histidine	$0.145^{a} \pm 0.010$	$0.185^{a} \pm 0.030$	$0.166^{a} \pm 0.020$	0.36
Anserine	$0.213^{a} \pm 0.040$	$0.202^{a} \pm 0.030$	$0.156^{a} \pm 0.030$	0.39
Carnosine	$0.049^{a} \pm 0.007$	$0.042^{a} \pm 0.009$	$0.059^{a} \pm 0.020$	0.66
Longissimus Lumborum				
Histidine	$0.049^{a} \pm 0.010$	$0.076^{b} \pm 0.010$	$0.051^{a} \pm 0.006$	**0.04***
Anserine	$0.149^{a} \pm 0.010$	$0.141^{a} \pm 0.010$	$0.133^{a} \pm 0.009$	0.63
Carnosine	$0.010^{a} \pm 0.0004$	$0.010^{a} \pm 0.0007$	$0.010^{a} \pm 0.0007$	0.87

Free HIS, ANS, and CARN content within muscle tissues of steers (N=48) supplemented with RP HIS during finishing. Tissues were derivatized and evaluated using HPLC for metabolite content. The low HIS LL steaks contained significantly higher free HIS than either other treatment. No other differences were detected in metabolite content.

* P-values show statisical differences

§ Values indicate mean mg/g wet tissue weight basis

[1] No histidine supplementation, N=16

[2] 50 g/hd/d histidine supplementation, N=16

[3] 100 g/hd/d histidine supplementation, N=16

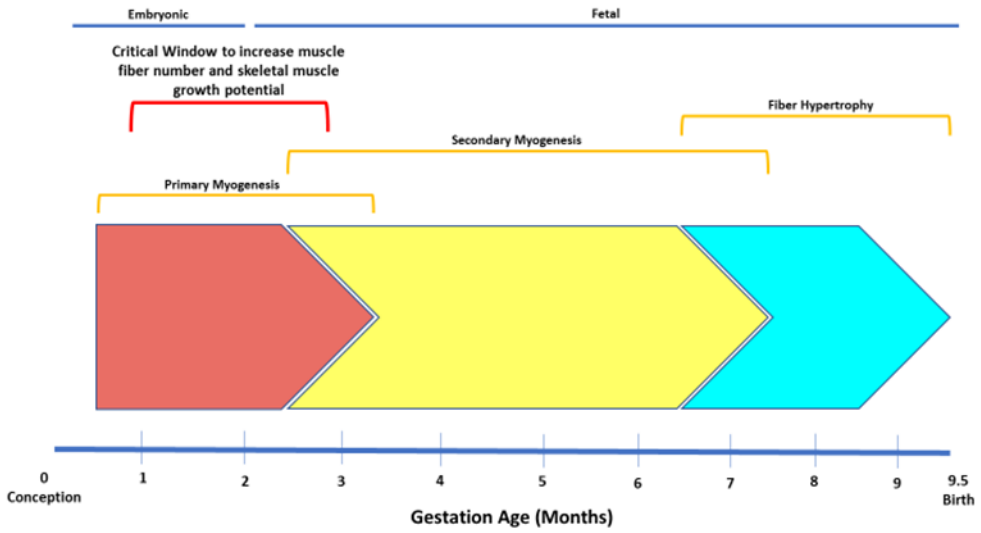


Figure 1.1. Embryonic and fetal critical windows for skeletal muscle growth. (Adapted from Du et al., 2010)

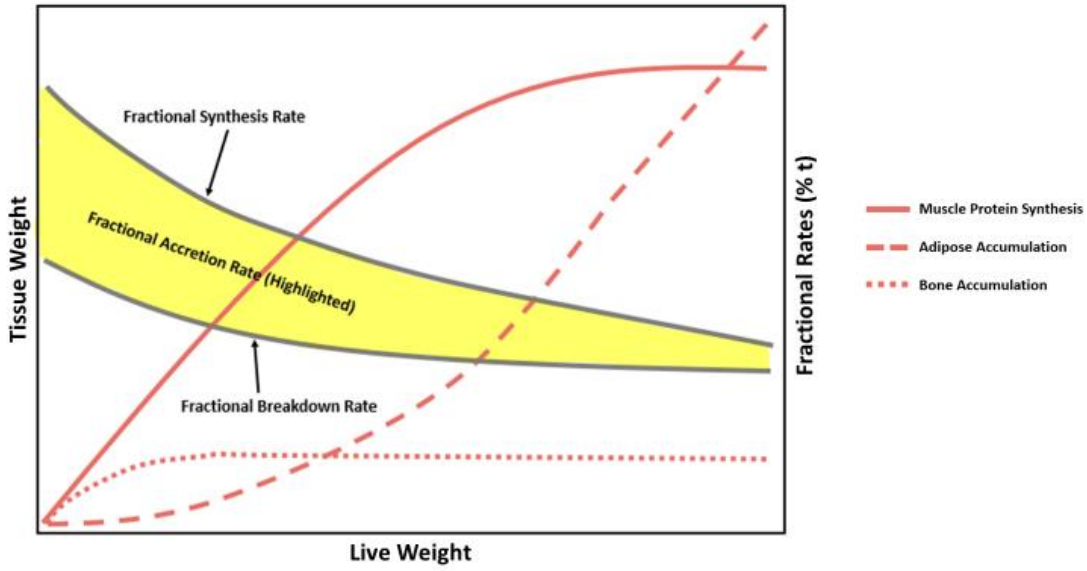


Figure 1.2. Fractional accretion rate as it relates to bone, muscle, and adipose tissue accretion. (Adapted from Kumar et al., 1999; and Goll et al., 2008)

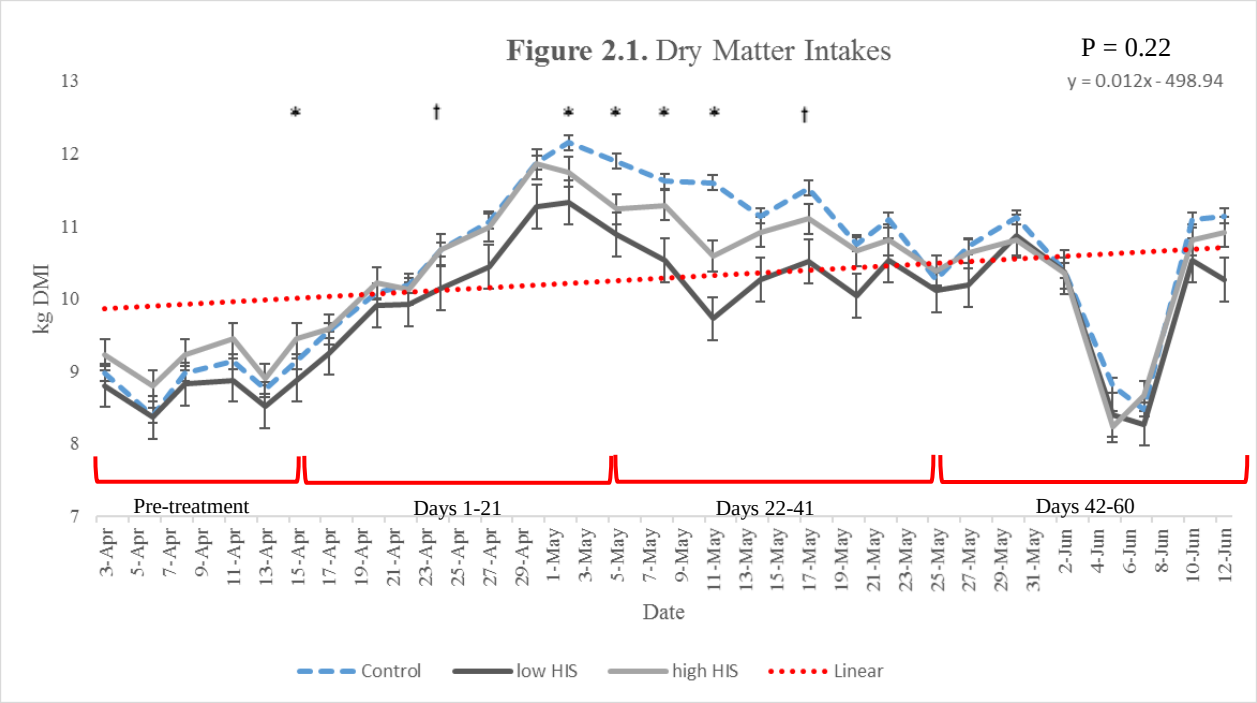


Figure 2.1. Daily dry matter intakes of steers (N=48) over finishing period. No difference (P > 0.10) was seen in average daily intake across treatments.

* Indicates day where treatment difference detected P ≤ 0.05

† Indicates day where treatment tendency detected P ≤ 0.10

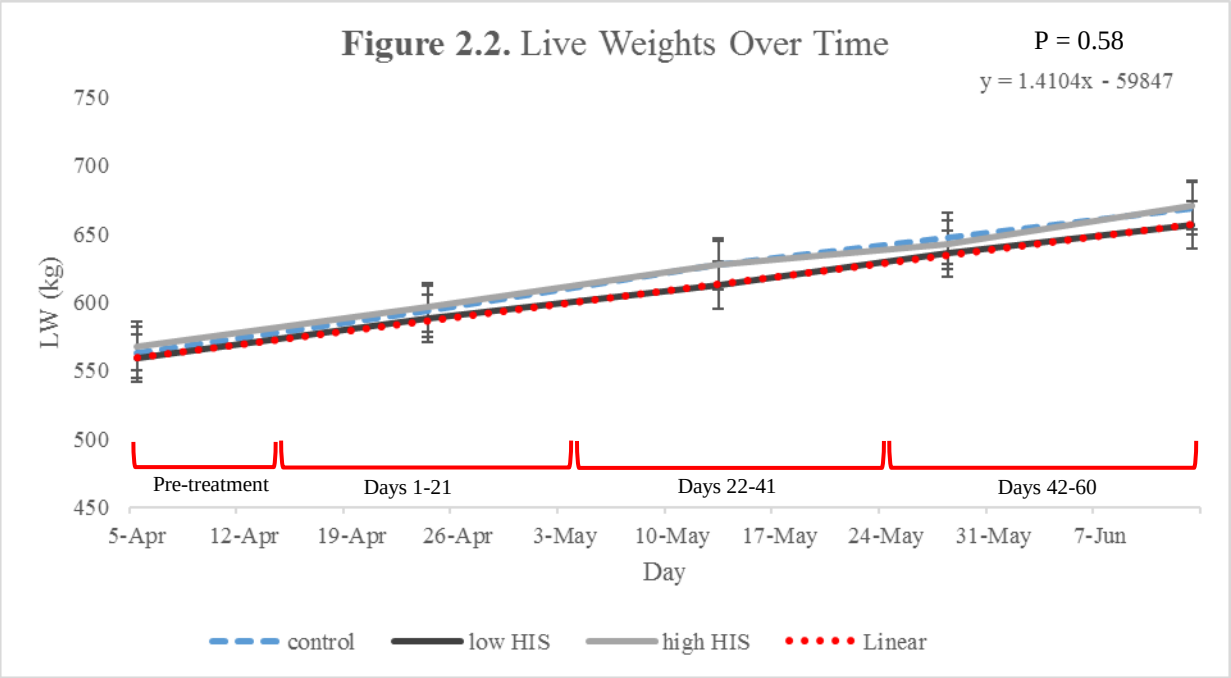


Figure 2.2. Live weight gain over time. Animals (N=48) were weighted approximately bi-weekly. No difference (P > 0.10) was seen in average live weight across treatments.

* Indicates day where P ≤ 0.05

† Indicates day where P ≤ 0.10

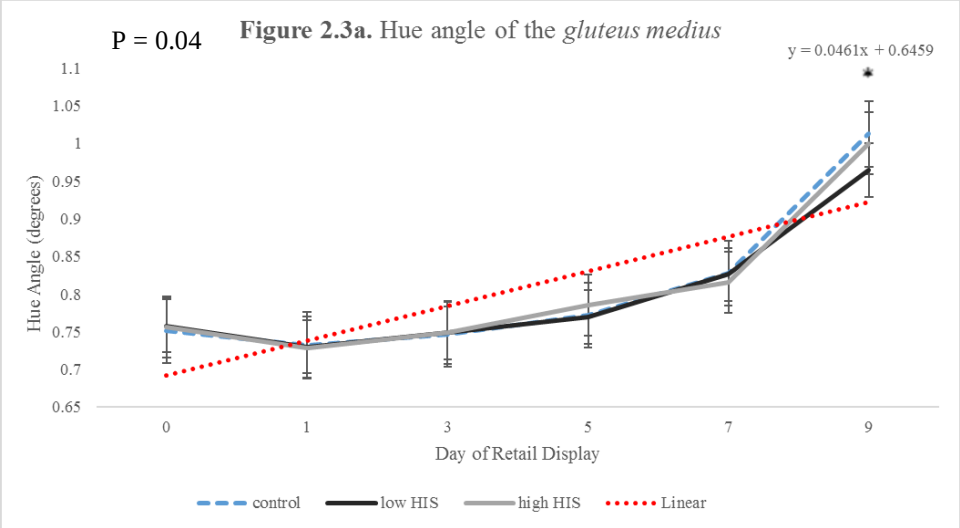


Figure 2.3a. Hue angle over 9 day simulated retail display. Hue angle was calculated using values from instramental color measurements. Supplementation with RP HIS during the final 55 days of finishing yielded decreased hue angle values for the low HIS product compared to control (P = 0.005) as well as high HIS (P = 0.04).

* Indicates day where P ≤ 0.05

† Indicates day where P ≤ 0.10

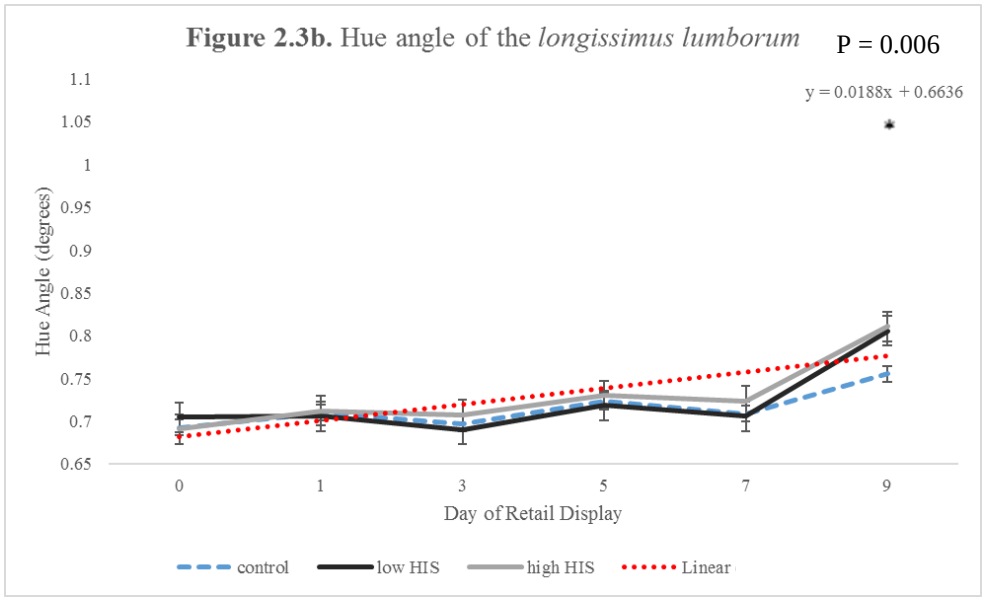


Figure 2.3b. Hue angle over 9 day simulated retail display. Hue angle was calculated using values from instramental color measurements. Increased hue angle values were observed on day 9 of retail display in both the low HIS (P = 0.006) and high HIS (P = 0.003) treatment groups.

* Indicates day where P ≤ 0.05

† Indicates day where P ≤ 0.10

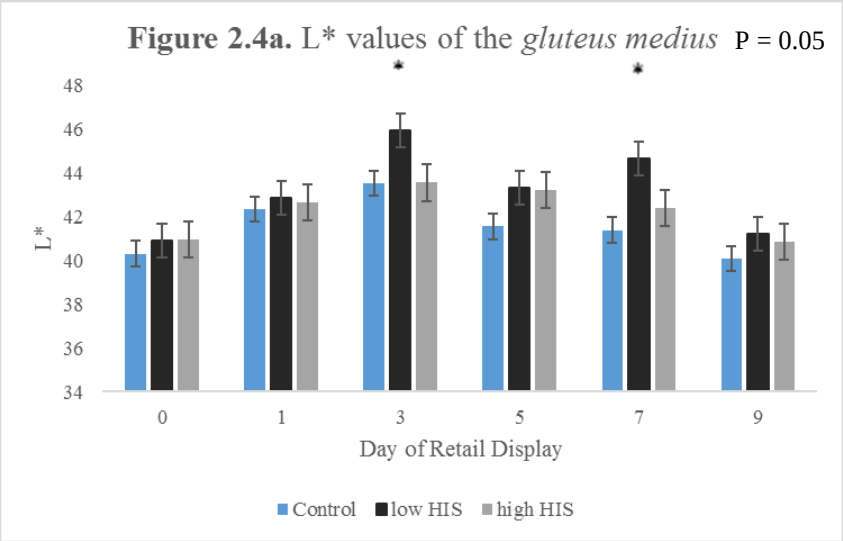


Figure 2.4a. L* brightness values over 9 day simulated retail display taken with Hunter MiniScan EZ in repeated measures. Supplementation with histidine increased the L* values for the low HIS GM product on days 3 (P = 0.03) and 7 (P = 0.003) of retail display in comparison to the control steaks and high HIS steaks. A treatment effect was observed for the low HIS GM product throughout the retail display period (P = 0.05),

* Indicates day where P ≤ 0.05

† Indicates day where P ≤ 0.10

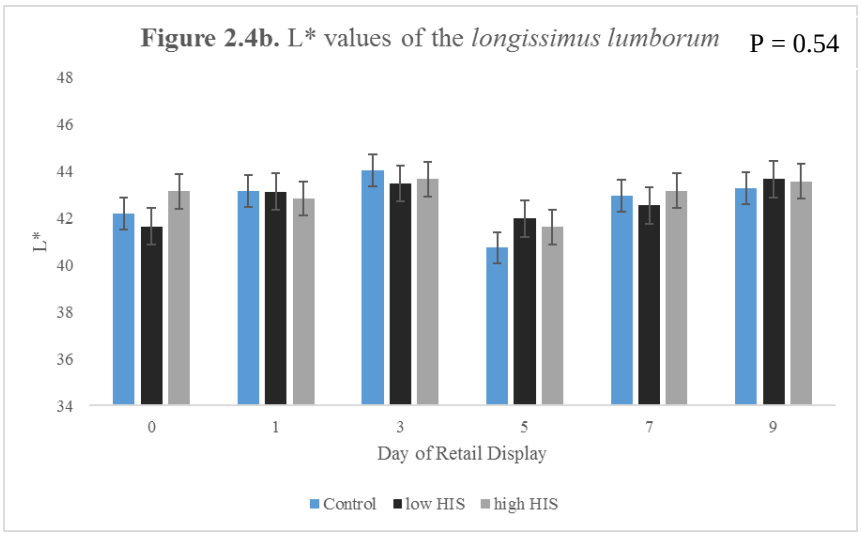


Figure 2.4a. L* brightness values over 9 day simulated retail display taken with Hunter MiniScan EZ in repeated measures. Supplementation with histidine yeilded no effects on L* value of the *longissimus lumborum.*

* Indicates day where P ≤ 0.05

† Indicates day where P ≤ 0.10

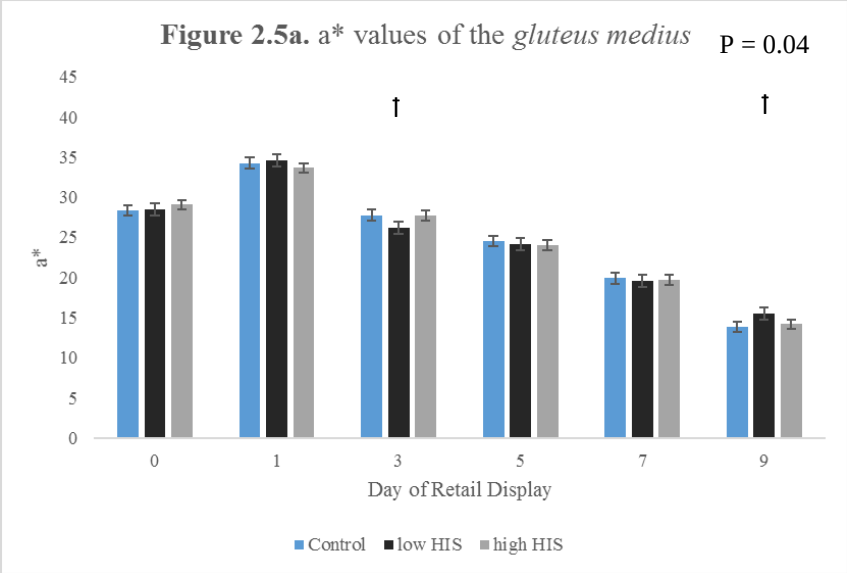


Figure 2.5a. a* red/green values over 9 day simulated retail display taken with Hunter MiniScan EZ in repeated measures. Muscles were aged for 14 days and cut into 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 9 days. An interaction between days and treatment (P = 0.04) was observed in the GM product for a* (red/green), yielding redder lean color for the low HIS product. Additionally, a trend for lower a* values were observed for the low HIS group over high HIS and control on day 3 of retail display (P = 0.08) as well as higher a* values on day 9 (P = 0.07)

* Indicates day where P ≤ 0.05

† Indicates day where P ≤ 0.10

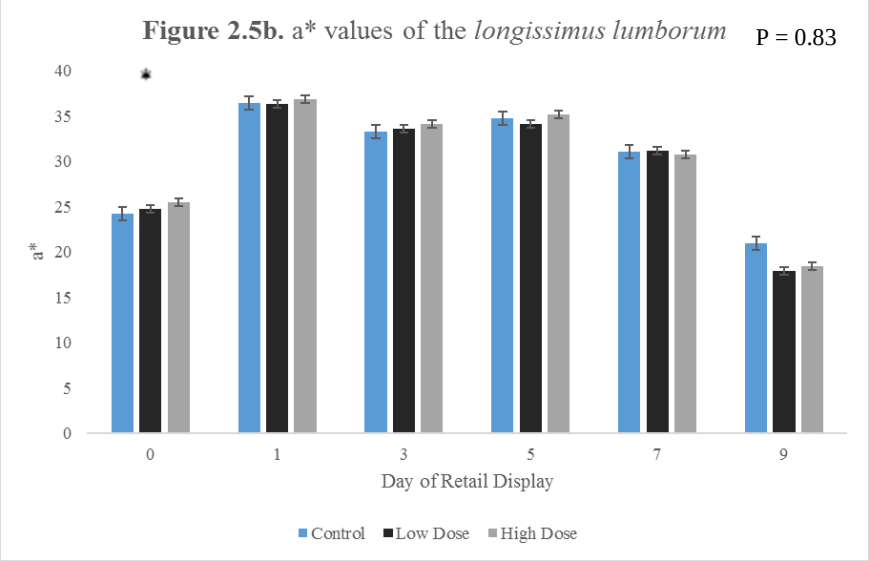


Figure 2.5b. a* red/green values over 9 day simulated retail display taken with Hunter MiniScan EZ in repeated measures. Muscles were aged for 21 days and cut into 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 9 days.The high HIS LL steaks had higher a* values than control on day 0 of retail display, indicating redder lean pigment (P = 0.05).

* Indicates day where P ≤ 0.05

† Indicates day where P ≤ 0.10

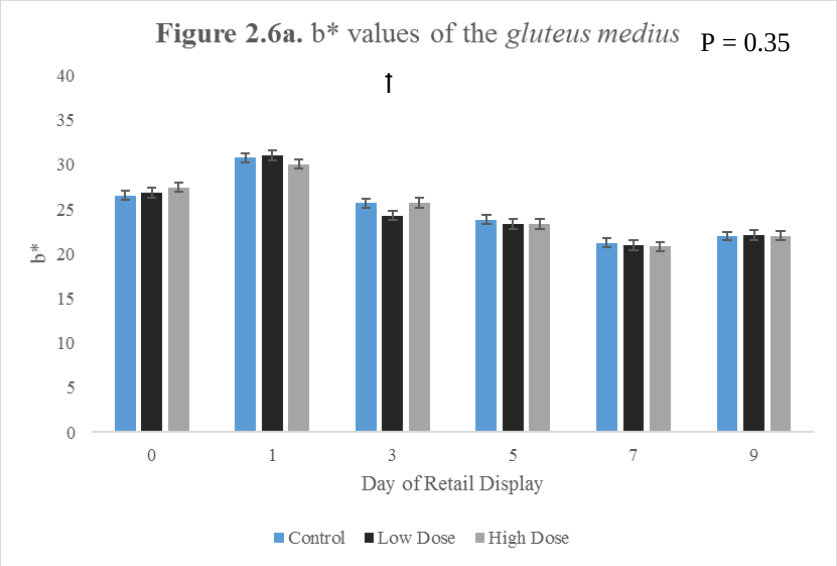


Figure 2.6a. b* yellow/blue values over 9 day simulated retail display taken with Hunter MiniScan EZ in repeated measures. Muscles were aged for 14 days and cut into 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 9 days. A trend was observed for lower b* value in the low HIS steaks on day 3 of retail display indicating a higher degree of blue pigment (P = 0.06).

* Indicates day where P ≤ 0.05

† Indicates day where P ≤ 0.10

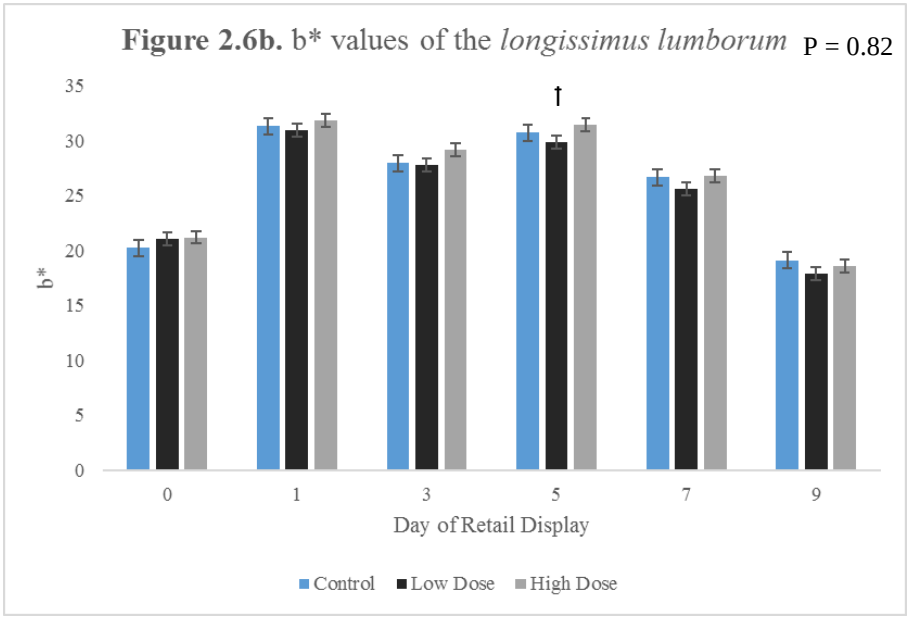


Figure 2.6b. b* yellow/blue values over 9 day simulated retail display taken with Hunter MiniScan EZ in repeated measures. Muscles were aged for 21 days and cut into 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 9 days. The b* value tended to be lower for the low HIS LL treatment on day 5 of retail display (P = 0.06), indicating a tendency for an increase in blue pigment.

* Indicates day where P ≤ 0.05

† Indicates day where P ≤ 0.10

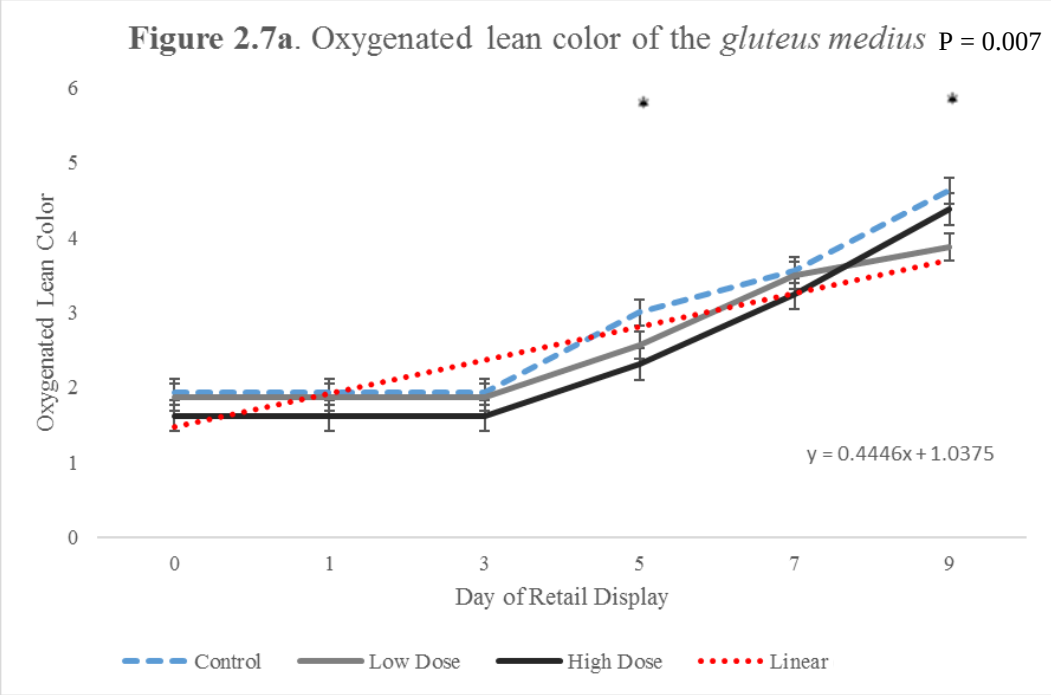


Figure 2.7a. Effects of histidine supplementation and retail display on oxygenated lean color. Muscles were aged for 14 days and cut into 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 9 days. Two evaluators scored the steaks on days 0,1,3,5,7, and 9 during retail display for oxygenated lean color using an 8 point scale (1 = extremely bright cherry-red, 2 = bright cherry-red, 3 = moderately bright cherry-red, 4 = slightly bright cherry-red, 5 = slightly dark cherry-red, 6 = moderately dark red, 7 = dark red, 8 = Extremely dark red). The low HIS group of GM steaks had significantly more oxygenated lean color (youthful bright cherry red) than control and the high HIS group on days 5 ($P = 0.009$) and 9 ($P = 0.004$) of retail display. The high HIS GM product was brighter cherry red than the control product on day 5 of retail display ($P = 0.05$).

* Indicates day where $P \leq 0.05$

† Indicates day where $P \leq 0.10$

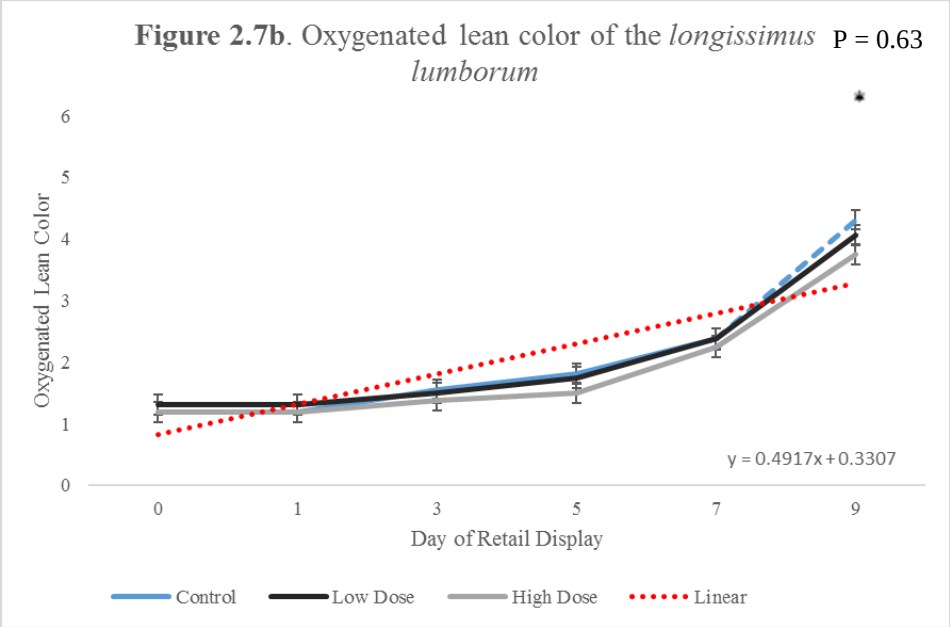


Figure 2.7b. Effects of histidine supplementation and retail display on oxygenated lean color. Muscles were aged for 21 days and cut into 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 9 days. Two evaluators scored the steaks on days 0,1,3,5,7, and 9 during retail display for oxygenated lean color using an 8 point scale (1 = extremely bright cherry-red, 2 = bright cherry-red, 3 = moderately bright cherry-red, 4 = slightly bright cherry-red, 5 = slightly dark cherry-red, 6 = moderately dark red, 7 = dark red, 8 = Extremely dark red). The high HIS LL product proved to have brighter cherry red lean color values on day 9 (P = 0.01) of retail display compared to control. On average, scores increased by 0.46 units per day of retail display.

* Indicates day where $P \leq 0.05$

† Indicates day where $P \leq 0.10$

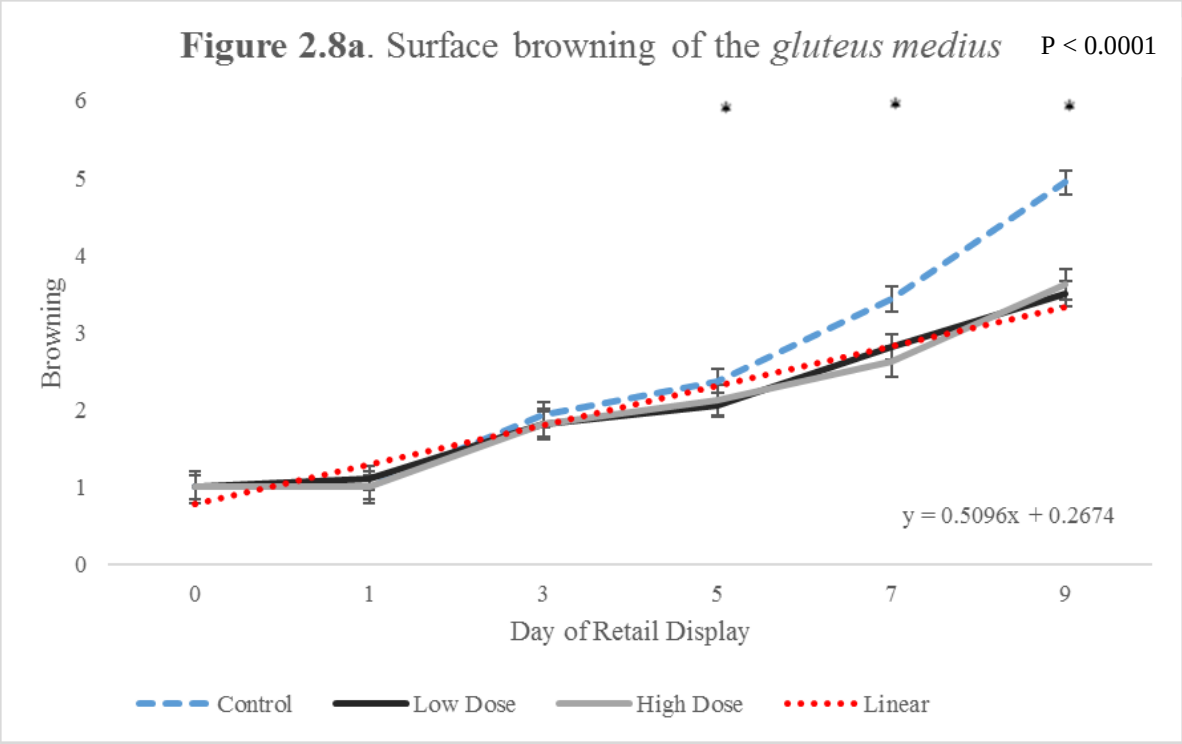


Figure 2.8a. Effects of histidine supplementation and retail display on amount of browning of beef *gluteus medius*. Muscles were aged for 14 days and cut into 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 9 days. Two evaluators scored the steaks on days 0,1,3,5,7, and 9 during retail display for amount of browning using a 6 point scale (1 = no evidence of browning, 2 = dull, 3 = grayish, 4 = brownish-gray, 5 = brown, 6 = dark brown). In the GM steaks, a treatment effect (P = 0.002) as well as an interaction between treatment and days (P < 0.0001) was observed for decreased browning on the surface of the product. On days 7 (P = 0.0002) and 9 (P < 0.0001), low HIS treatment had significantly less surface browning than control in the GM steaks. The same was observed for the high HIS group in comparison to control (d 5: P = 0.0002; d 9: P < 0.0001). On average, scores increased by 0.51 units per day of retail display.

* Indicates day where P ≤ 0.05

† Indicates day where P ≤ 0.10

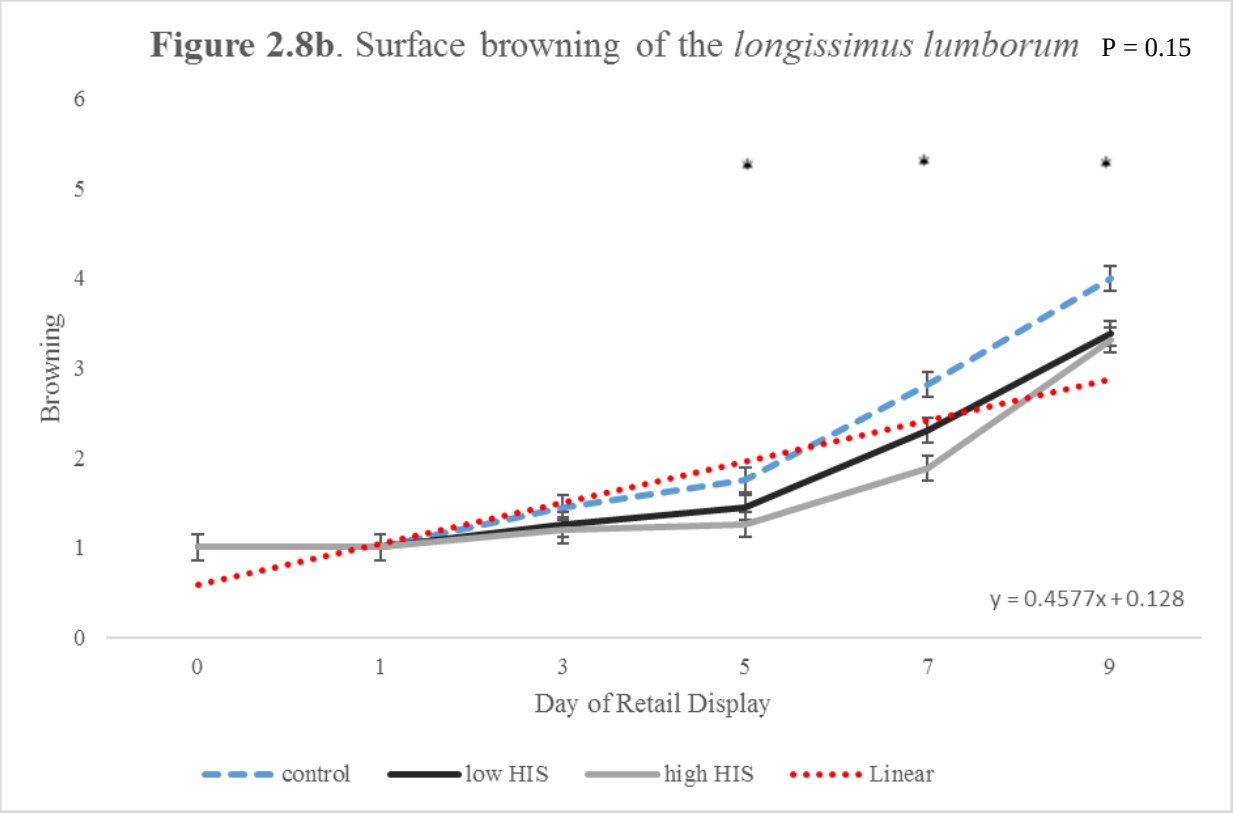


Figure 2.8b. Effects of histidine supplementation and retail display on amount of browning of beef *longissimus lumborum*. Muscles were aged for 21 days and cut into 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 9 days. Two evaluators scored the steaks on days 0,1,3,5,7, and 9 during retail display for amount of browning using a 6 point scale (1 = no evidence of browning, 2 = dull, 3 = grayish, 4 = brownish-gray, 5 = brown, 6 = dark brown). The low and high HIS LL steaks had significantly less browning on days 5 ($P = 0.02$), 7 ($P < 0.0001$) and 9 ($P = 0.001$) of retail display in comparison to control. On average, scores increased by 0.46 units per day.

* Indicates day where $P \leq 0.05$

† Indicates day where $P \leq 0.10$

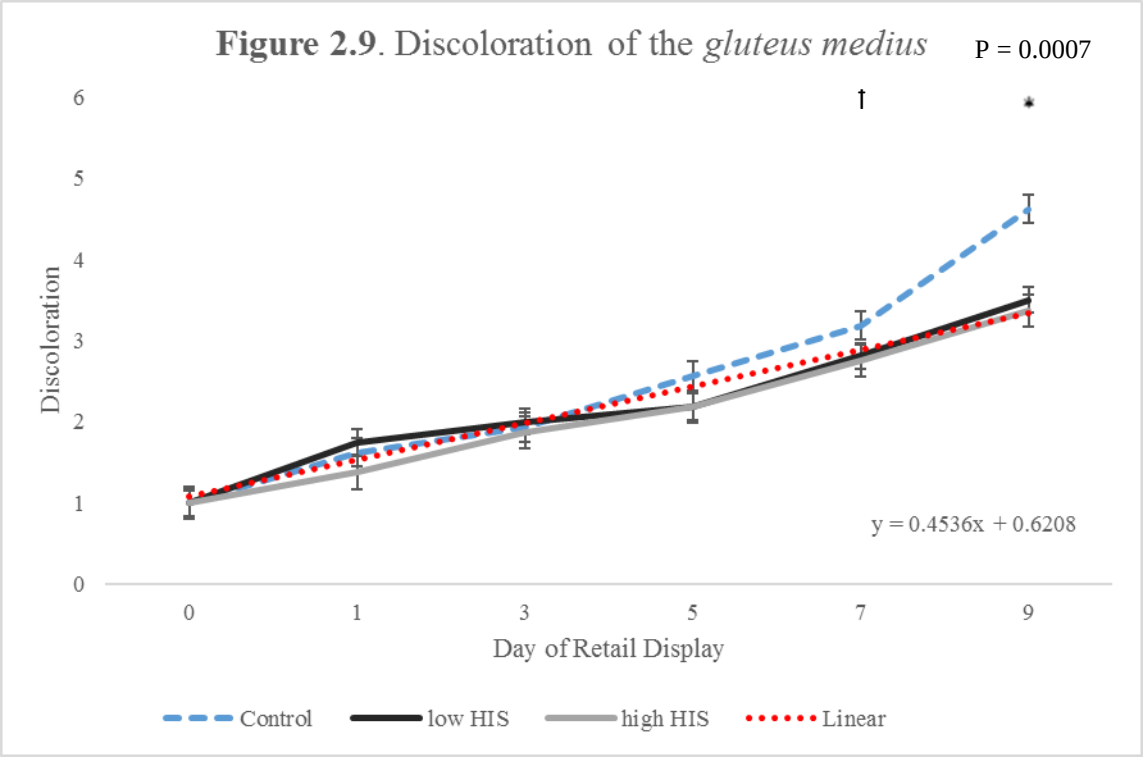


Figure 2.9a. Effects of histidine supplementation and retail display on discoloration of beef *gluteus medius*. Muscles were aged for 14 days and cut into 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 9 days. Two evaluators scored the steaks on days 0,1,3,5,7, and 9 during retail display for discoloration using a 5 point scale (1 = none, 2 = slight, 3 = small, 4 = moderate, 5 = extreme). Both the low HIS and high HIS steaks were significantly less discolored than the control on 9 ($P < 0.0001$) of retail display in the GM product. Additionally, the high HIS GM steaks tended to be less discolored than the low HIS group on day 7 ($P = 0.06$). On average scores increased by 0.45 units per day of display.

* Indicates day where $P \leq 0.05$

† Indicates day where $P \leq 0.10$

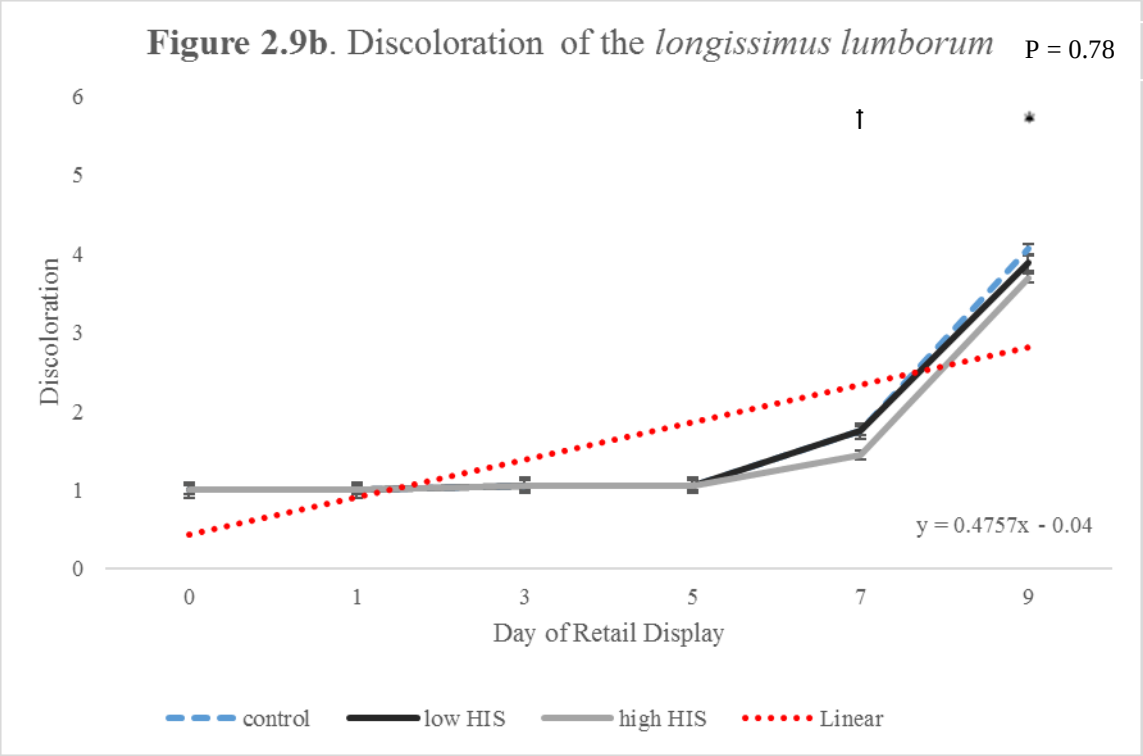


Figure 2.9b. Effects of histidine supplementation and retail display on discoloration of beef *longissimus lumborum*. Muscles were aged for 21 days and cut into 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 9 days. Two evaluators scored the steaks on days 0,1,3,5,7, and 9 during retail display for discoloration using a 5 point scale (1 = none, 2 = slight, 3 = small, 4 = moderate, 5 = extreme). The high HIS steaks from the LL group tended to be less discolored than control and low HIS on day 7 (P = 0.10) and were found to be significantly less discolored than control on day 9 (P = 0.04) of retail display. On average, sccores increased by 0.48 units per day of retail display.

* Indicates day where $P \leq 0.05$

† Indicates day where $P \leq 0.10$

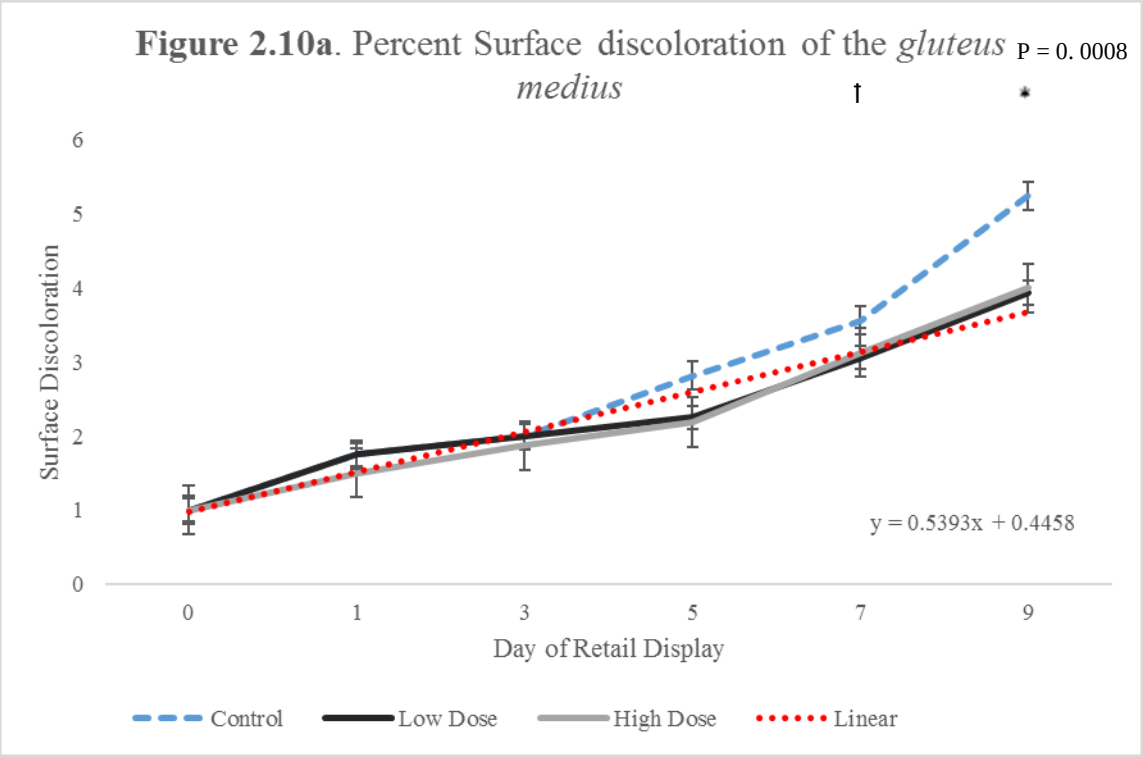


Figure 2.10a. Effects of histidine supplementation and retail display on % surface discoloration of beef *gluteus medius*. Muscles were aged for 14 days and cut into 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 9 days. Two evaluators scored the steaks on days 0,1,3,5,7, and 9 during retail display for percent surface discoloration using a 6 point scale (1 = none (0%), 2 = slight (1-20%), 3 = small (21-40%), 4 = modest (41-60%), 5 = moderate (61-80%), 6 = extensive (81-100%)). A low HIS treatment effect (P = 0.01) as well as a day by treatment interaction (P = 0.0008) was observed for percent surface discoloration (% metmyoglobin) in the GM product. On day 7, the high HIS GM steaks tended to have less % metmyoglobin than the low HIS and control (P = 0.06) and both treatments proved to have less discoloration than control on day 9 (P < 0.0001). On average, scores inreased by 0.54 units per day of display.

* Indicates day where $P \leq 0.05$

† Indicates day where $P \leq 0.10$

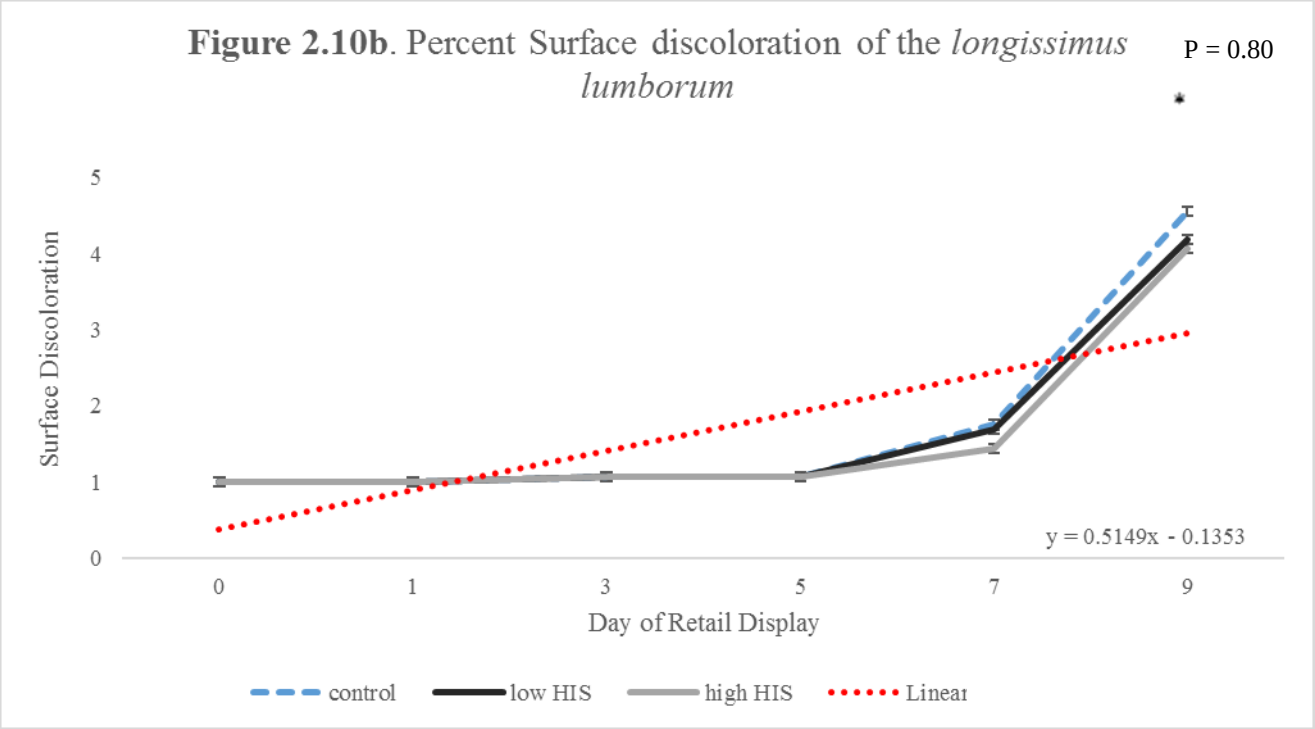


Figure 2.10b. Effects of histidine supplementation and retail display on discoloration of beef *longissimus lumborum*. Muscles were aged for 21 days and cut into 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 9 days. Two evaluators scored the steaks on days 0,1,3,5,7, and 9 during retail display for percent surface discoloration using a 6 point scale (1 = none (0%), 2 = slight (1-20%), 3 = small (21-40%), 4 = modest (41-60%), 5 = moderate (61-80%), 6 = extensive (81-100%)). A decrease in metmyoglobin percentage was seen in the high HIS LL product over the control (P = 0.03) on day 9 of retail display. On average, scores increased by 0.52 units per day of display.

* Indicates day where $P \leq 0.05$

† Indicates day where $P \leq 0.10$

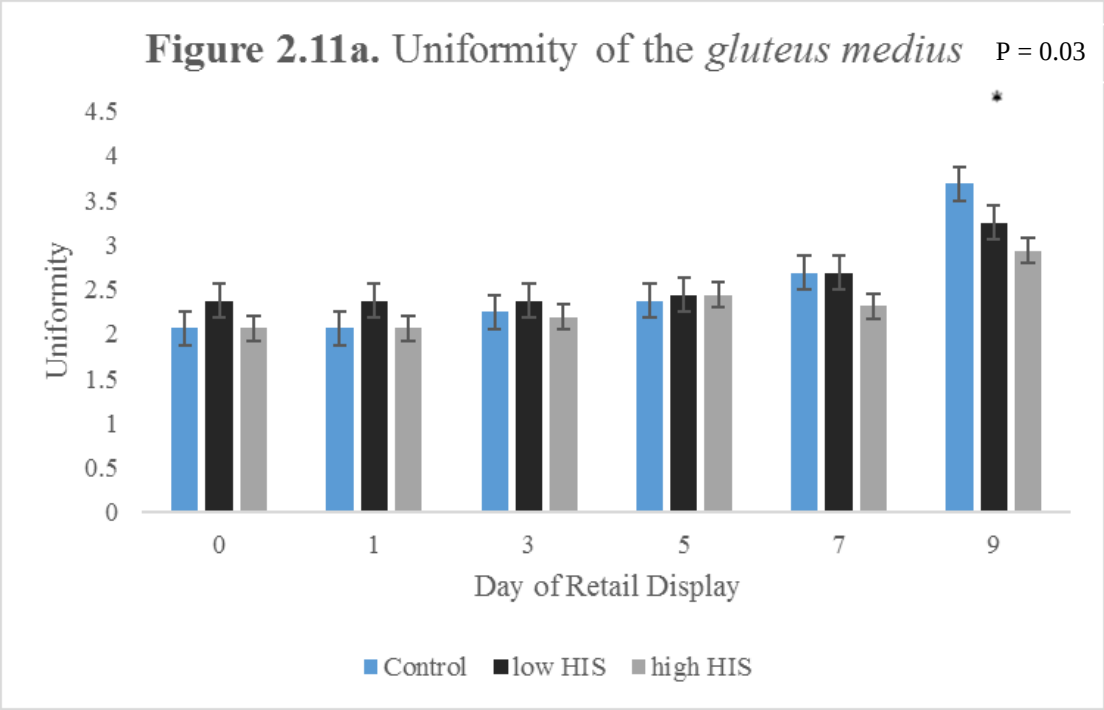


Figure 2.11a. Effects of histidine supplementation and retail display on uniformity of beef *gluteus medius*. Muscles were aged for 14 days and cut into 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 9 days. Two evaluators scored the steaks on days 0,1,3,5,7, and 9 during retail display for color uniformity using a 5 point scale (1 = uniform, 2 = slight two-toning, 3 = small amount of two-toning, 4 = moderate two-toning, 5 = extreme two-toning). A day by treatment interaction (P = 0.03) was observed for color uniformity in the GM product throughout retail display. On day 9, the high and low HIS GM steaks were more uniform than control (P = 0.001).

* Indicates day where $P \leq 0.05$

† Indicates day where $P \leq 0.10$

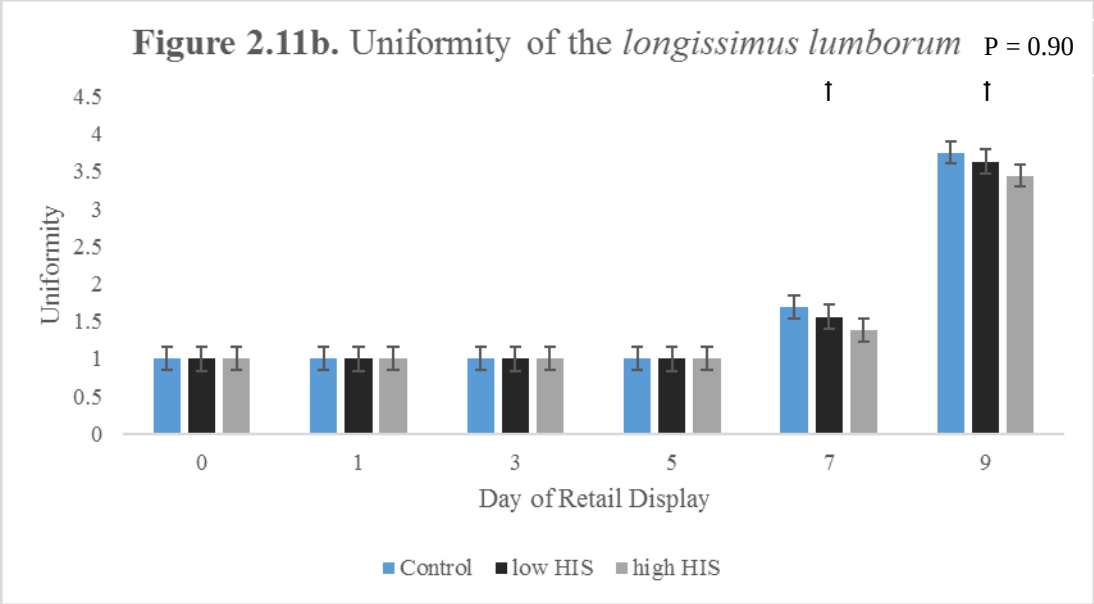


Figure 2.11b. Effects of histidine supplementation and retail display on discoloration of beef *longissimus lumborum*. Muscles were aged for 21 days and cut into 2.54 cm-thick steaks were cut from designated sections and displayed in a glass-fronted retail display case at 3°C for 9 days. Two evaluators scored the steaks on days 0,1,3,5,7, and 9 during retail display for color uniformity using a 5 point scale (1 = uniform, 2 = slight two-toning, 3 = small amount of two-toning, 4 = moderate two-toning, 5 = extreme two-toning). On days 7 ($P = 0.06$) and 9 ($P = 0.06$), the high HIS LL steaks tended to be more uniform than control.

* Indicates day where $P \leq 0.05$

† Indicates day where $P \leq 0.10$

Figure 2.12a-2.12b.

Effects of histidine supplementation on free histidine, anserine, and carnosine content within the *gluteus medius* and *longissimus lumborum*

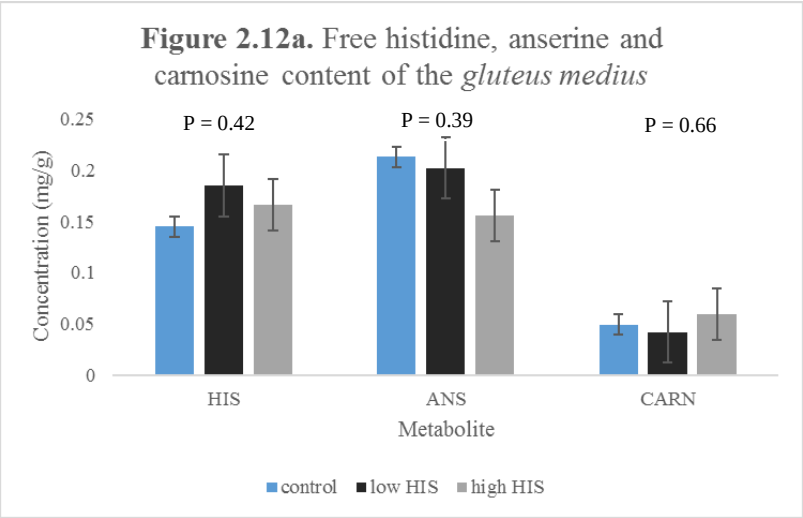


Figure 2.12a. Effects of histidine supplementation and retail display on the free histidine, anserine, and carnosine contents of beef gluteus medius. Tissue samples were snap frozen, derivatized, and analyzed using HPLC. No difference was observed in free metabolite content ($P > 0.10$).

* Indicates day where $P \leq 0.05$

† Indicates day where $P \leq 0.10$

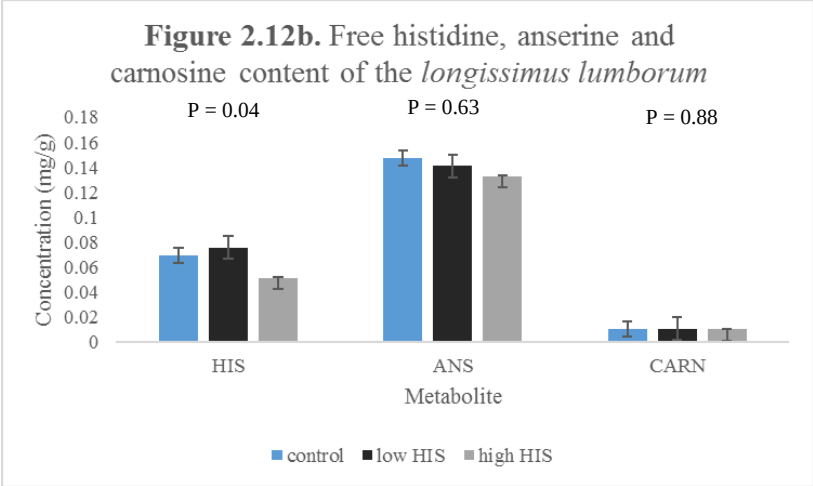


Figure 2.12b. Effects of histidine supplementation and retail display on the free histidine, anserine, and carnosine contents of beef longissimus lumborum. Tissue samples were snap frozen, derivatized, and analyzed using HPLC. The low HIS product had higher free HIS content than both the high HIS and control groups ($P = 0.04$). No difference was observed in ANS or CARN content among treatments.

* Indicates day where $P \leq 0.05$

† Indicates day where $P \leq 0.10$

Appendix A

Exempt Certification for IRB Project Number 16-068

University of Idaho
Office of Research Assurances
Institutional Review Board
875 Perimeter Drive, MS 3010
Moscow ID 83844-3010
Phone: 208-885-6162
Fax: 208-885-5752
irb@uidaho.edu

To:	Gordon K. Murdoch
Cc:	Matthew E. Doumit, Breann Sandberg
From:	Jennifer Walker, IRB Coordinator
Approval Date:	August 22, 2016
Title:	Year two: Improving color, color stability and flavor of the top sirloin through dietary rumen protected histidine supplementation.
Project:	16-068
Certified:	Certified as exempt under category 6 at 45 CFR 46.101(b)(6).

On behalf of the Institutional Review Board at the University of Idaho, I am pleased to inform you that the protocol for the research project "Year two: Improving color, color stability and flavor of the top sirloin through dietary rumen protected histidine supplementation" has been certified as exempt under the category and reference number listed above.

This certification is valid only for the study protocol as it was submitted. Studies certified as Exempt are not subject to continuing review and this certification does not expire. However, if changes are made to the study protocol, you must submit the changes through VERAS for review before implementing the changes. Amendments may include but are not limited to, changes in study population, study personnel, study instruments, consent documents, recruitment materials, sites of research, etc. If you have any additional questions, please contact me through the VERAS messaging system by clicking the 'Reply' button.

As Principal Investigator, you are responsible for ensuring compliance with all applicable FERPA regulations, University of Idaho policies, state and federal regulations. Every effort should be made to ensure that the project is conducted in a manner consistent with the three fundamental principles identified in the Belmont Report: respect for persons; beneficence; and justice. The Principal Investigator is responsible for ensuring that all study personnel have completed the online human subjects training requirement.

You are required to timely notify the IRB if any unanticipated or adverse events occur during the study, if you experience and increased risk to the participants, or if you have participants withdraw or register complaints about the study.

Appendix B

Feed Sample Nutrient Analysis

(Cumberland Valley Analytics)

Appendix B. Feed sample nutrient analysis for grab samples 1-11

	Mean	Range
Chemistry, Energy, Index		
% DM	70.00	63-80
Neg (Mcal/lb)	0.57	0.53-0.59
CP (%DM)	18.7	17.3-20.7
eNDF (%DM)	24.3	21.7-29.5
ADF (%DM)	12.9	10.3-18.7
NFC (%DM)	57.4	52.6-60.8
Minerals		
Calcium (%DM)	0.8	0.54-1.23
Phosphorus (%DM)	0.42	0.40-0.45
Potassium (%DM)	0.92	0.75-1.19
Magnesium (%DM)	0.21	0.20-0.22
Sodium (%DM)	0.21	0.16-0.23
Iron (PPM)	283	232-440
Manganese (PPM)	57	47-70
Zinc (PPM)	87	70-107
Copper (PPM)	19	15-27

DM: Dry Matter

Neg: Net energy growth

eNDF: Neutral detergent fiber

ADF: Acid detergent fiber

NFC: Non-fiber carbohydrates

Appendix B: Chemical Analysis of TMR (Cumberland Valley Analytics). Values reported are the mean and range of all grab samples analyzed.

Appendix C

Total Mixed Ration; Ingredient Formulation

C1-C7

Appendix C1. Total Mixed Ration (Final 55 d)

Ingredient	DM Content (%)	Ration DMB (%)
Hay, alfalfa	89	8
DDG	92	18
Apples	2	11
Corn	89	25
Barley	91	34
Performix Liquid Supplement (Bunk Start)	72	0
Performix Liquid Supplement	72	4

DDG: Dry distillers grains

Apples: apple pomace byproduct

Appendix C2. Total Mixed Ration (Step 1)

Ingredient	DM Content (%)	Ration DMB (%)
Hay, alfalfa	89	48
DDG	92	8
Apples	2	8
Corn	89	12
Barley	91	22
Performix Liquid Supplement (Bunk Start)	72	2
Performix Liquid Supplement	72	0

DDG: Dry distillers grains

Apples: apple pomace byproduct

Appendix C3. Total Mixed Ration (Step 2)

Ingredient	DM Content (%)	Ration DMB (%)
Hay, alfalfa	89	40
DDG	92	9
Apples	2	8
Corn	89	14
Barley	91	24
Performix Liquid Supplement (Bunk Start)	72	5
Performix Liquid Supplement	72	0

DDG: Dry distillers grains

Apples: apple pomace byproduct

Appendix C4. Total Mixed Ration (Step 3)

Ingredient	DM Content (%)	Ration DMB (%)
Hay, alfalfa	89	30
DDG	92	10
Apples	2	9
Corn	89	18
Barley	91	26.5
Performix Liquid Supplement (Bunk Start)	72	4
Performix Liquid Supplement	72	2.5

DDG: Dry distillers grains

Apples: apple pomace byproduct

Appendix C5. Total Mixed Ration (Step 4)

Ingredient	DM Content (%)	Ration DMB (%)
Hay, alfalfa	89	21
DDG	92	13
Apples	2	9
Corn	89	23
Barley	91	27.5
Performix Liquid Supplement (Bunk Start)	72	3
Performix Liquid Supplement	72	3.5

DDG: Dry distillers grains

Apples: apple pomace byproduct

Appendix C6. Total Mixed Ration (Step 5)

Ingredient	DM Content (%)	Ration DMB (%)
Hay, alfalfa	89	11
DDG	92	18
Apples	2	11
Corn	89	22
Barley	91	34
Performix Liquid Supplement (Bunk Start)	72	0
Performix Liquid Supplement	72	4

DDG: Dry distillers grains

Apples: apple pomace byproduct

Appendix C7. Total Mixed Ration (Step 6)

Ingredient	DM Content (%)	Ration DMB (%)
Hay, alfalfa	89	13
DDG	92	18
Apples	2	11
Corn	89	22
Barley	91	32
Performix Liquid Supplement (Bunk Start)	72	0
Performix Liquid Supplement	72	4

Daily histidine provision was estimated at 251% requirements using Beef Cattle Nutrient Requirement Model (NRC, 2016). Histidine is also estimated as the 1st limiting amino acid in this ration.

DDG: Dry distillers grains

Apples: apple pomace byproduct

Appendix D

Feed Sample Chemical Analysis for Grab Samples 1-11

(Cumberland Valley Analytics)

Appendix D1. Feed sample nutrient analysis for grab sample 1

	Sample 1
Chemistry, Energy, Index	
% DM	72.00
Neg (Mcal/lb)	0.57
CP (%DM)	17.3
eNDF (%DM)	24.5
ADF (%DM)	12
NFC (%DM)	58.2
Minerals	
Calcium (%DM)	0.93
Phosphorus (%DM)	0.42
Potassium (%DM)	0.84
Magnesium (%DM)	0.21
Sodium (%DM)	0.26
Iron (PPM)	330
Manganese (PPM)	70
Zinc (PPM)	107
Copper (PPM)	22

DM: Dry Matter

Neg: Net energy growth

eNDF: Neutral detergent fiber

ADF: Acid detergent fiber

NFC: Non-fiber carbohydrates

Appendix D2. Feed sample nutrient analysis for grab sample 2

	Sample 2
Chemistry, Energy, Index	
% DM	70.00
Neg (Mcal/lb)	0.57
CP (%DM)	20.7
eNDF (%DM)	23.8
ADF (%DM)	12.5
NFC (%DM)	55.5
Minerals	
Calcium (%DM)	1.23
Phosphorus (%DM)	0.45
Potassium (%DM)	0.9
Magnesium (%DM)	0.22
Sodium (%DM)	0.23
Iron (PPM)	440
Manganese (PPM)	68
Zinc (PPM)	105
Copper (PPM)	27

DM: Dry Matter

Neg: Net energy growth

eNDF: Neutral detergent fiber

ADF: Acid detergent fiber

NFC: Non-fiber carbohydrates

Appendix D3. Feed sample nutrient analysis for grab sample 3

	Sample 3
Chemistry, Energy, Index	
% DM	66.30
Neg (Mcal/lb)	0.57
CP (%DM)	17.3
eNDF (%DM)	23.6
ADF (%DM)	11.5
NFC (%DM)	59.2
Minerals	
Calcium (%DM)	0.72
Phosphorus (%DM)	0.41
Potassium (%DM)	0.89
Magnesium (%DM)	0.2
Sodium (%DM)	0.2
Iron (PPM)	274
Manganese (PPM)	56
Zinc (PPM)	86
Copper (PPM)	18

DM: Dry Matter

Neg: Net energy growth

eNDF: Neutral detergent fiber

ADF: Acid detergent fiber

NFC: Non-fiber carbohydrates

Appendix D4. Feed sample nutrient analysis for grab sample 4

	Sample 4
Chemistry, Energy, Index	
% DM	79.50
Neg (Mcal/lb)	0.58
CP (%DM)	17.40
eNDF (%DM)	21.80
ADF (%DM)	10.30
NFC (%DM)	60.80
Minerals	
Calcium (%DM)	0.78
Phosphorus (%DM)	0.41
Potassium (%DM)	0.75
Magnesium (%DM)	0.21
Sodium (%DM)	0.21
Iron (PPM)	221
Manganese (PPM)	52
Zinc (PPM)	80
Copper (PPM)	17

DM: Dry Matter

Neg: Net energy growth

eNDF: Neutral detergent fiber

ADF: Acid detergent fiber

NFC: Non-fiber carbohydrates

Appendix D5. Feed sample nutrient analysis for grab sample 5

	Sample 5
Chemistry, Energy, Index	
% DM	72.30
Neg (Mcal/lb)	0.56
CP (%DM)	18.80
eNDF (%DM)	24.60
ADF (%DM)	14.10
NFC (%DM)	56.60
Minerals	
Calcium (%DM)	0.64
Phosphorus (%DM)	0.41
Potassium (%DM)	1.00
Magnesium (%DM)	0.20
Sodium (%DM)	0.17
Iron (PPM)	233
Manganese (PPM)	47
Zinc (PPM)	70
Copper (PPM)	15

DM: Dry Matter

Neg: Net energy growth

eNDF: Neutral detergent fiber

ADF: Acid detergent fiber

NFC: Non-fiber carbohydrates

Appendix D6. Feed sample nutrient analysis for grab sample 6

	Sample 6
Chemistry, Energy, Index	
% DM	62.90
Neg (Mcal/lb)	0.53
CP (%DM)	17.90
eNDF (%DM)	29.50
ADF (%DM)	18.70
NFC (%DM)	52.60
Minerals	
Calcium (%DM)	0.62
Phosphorus (%DM)	0.45
Potassium (%DM)	1.19
Magnesium (%DM)	0.21
Sodium (%DM)	0.19
Iron (PPM)	262
Manganese (PPM)	51
Zinc (PPM)	79
Copper (PPM)	18

DM: Dry Matter

Neg: Net energy growth

eNDF: Neutral detergent fiber

ADF: Acid detergent fiber

NFC: Non-fiber carbohydrates

Appendix D7. Feed sample nutrient analysis for grab sample 7

	Sample 7
Chemistry, Energy, Index	
% DM	66.60
Neg (Mcal/lb)	0.59
CP (%DM)	17.90
eNDF (%DM)	22.80
ADF (%DM)	12.30
NFC (%DM)	59.20
Minerals	
Calcium (%DM)	0.97
Phosphorus (%DM)	0.43
Potassium (%DM)	0.85
Magnesium (%DM)	0.21
Sodium (%DM)	0.22
Iron (PPM)	308
Manganese (PPM)	61
Zinc (PPM)	90
Copper (PPM)	20

DM: Dry Matter

Neg: Net energy growth

eNDF: Neutral detergent fiber

ADF: Acid detergent fiber

NFC: Non-fiber carbohydrates

Appendix D8. Feed sample nutrient analysis for grab sample 8

	Sample 8
Chemistry, Energy, Index	
% DM	66.50
Neg (Mcal/lb)	0.59
CP (%DM)	20.00
eNDF (%DM)	22.90
ADF (%DM)	12.10
NFC (%DM)	57.10
Minerals	
Calcium (%DM)	0.95
Phosphorus (%DM)	0.44
Potassium (%DM)	0.86
Magnesium (%DM)	0.22
Sodium (%DM)	0.23
Iron (PPM)	311
Manganese (PPM)	65
Zinc (PPM)	98
Copper (PPM)	24

DM: Dry Matter

Neg: Net energy growth

eNDF: Neutral detergent fiber

ADF: Acid detergent fiber

NFC: Non-fiber carbohydrates

Appendix D9. Feed sample nutrient analysis for grab sample 9

	Sample 9
Chemistry, Energy, Index	
% DM	68.60
Neg (Mcal/lb)	0.57
CP (%DM)	17.80
eNDF (%DM)	21.70
ADF (%DM)	10.30
NFC (%DM)	60.50
Minerals	
Calcium (%DM)	0.80
Phosphorus (%DM)	0.40
Potassium (%DM)	0.80
Magnesium (%DM)	0.20
Sodium (%DM)	0.23
Iron (PPM)	269
Manganese (PPM)	62
Zinc (PPM)	91
Copper (PPM)	21

DM: Dry Matter

Neg: Net energy growth

eNDF: Neutral detergent fiber

ADF: Acid detergent fiber

NFC: Non-fiber carbohydrates

Appendix D10. Feed sample nutrient analysis for grab sample 10

	Sample 10
Chemistry, Energy, Index	
% DM	70.30
Neg (Mcal/lb)	0.56
CP (%DM)	18.30
eNDF (%DM)	26.90
ADF (%DM)	14.10
NFC (%DM)	54.90
Minerals	
Calcium (%DM)	0.54
Phosphorus (%DM)	0.43
Potassium (%DM)	1.01
Magnesium (%DM)	0.21
Sodium (%DM)	0.17
Iron (PPM)	232
Manganese (PPM)	48
Zinc (PPM)	76
Copper (PPM)	15

DM: Dry Matter

Neg: Net energy growth

eNDF: Neutral detergent fiber

ADF: Acid detergent fiber

NFC: Non-fiber carbohydrates

Appendix D11. Feed sample nutrient analysis for grab sample 11

	Sample 11
Chemistry, Energy, Index	
% DM	69.90
Neg (Mcal/lb)	0.56
CP (%DM)	18.10
eNDF (%DM)	25.40
ADF (%DM)	14.80
NFC (%DM)	56.40
Minerals	
Calcium (%DM)	0.57
Phosphorus (%DM)	0.42
Potassium (%DM)	1.00
Magnesium (%DM)	0.20
Sodium (%DM)	0.17
Iron (PPM)	241
Manganese (PPM)	48
Zinc (PPM)	73
Copper (PPM)	17

DM: Dry Matter

Neg: Net energy growth

eNDF: Neutral detergent fiber

ADF: Acid detergent fiber

NFC: Non-fiber carbohydrates

Appendix E

Taste Panel Evaluation Form and Release Form

SENSORY PANEL CONSENT FORM

1. The University of Idaho Human Assurance Committee has reviewed and found this study to be exempt.

2. The objective of this study was to evaluate the effects of extended aging and antioxidant treatment on beef. The samples will be prepared under the Research Guidelines for Cookery, Sensory Evaluation, and Instrument Tenderness Measurements of Fresh Meat, as outlined by the American Meat Science Association. This taste panel is part of research funded by the Idaho Beef Council.

3. You will be asked to evaluate 6 samples (approximately 1" x ½" x ½") per session for tenderness (1 = extremely tough to 9 = extremely tender), juiciness (1 = dry to 9 = juicy), and flavor (1 = bland to 9 = intense) using a 9 point scale. It is not necessary that samples be ingested. The study should take approximately 15 to 20 minutes.

4. Although there are no or minimal risks associated with the project, it is possible that some samples will have one or more qualities that may not be appealing to you (e.g. tenderness or juiciness that is less than you would prefer).

5. With your help, society can benefit from our attempt to improve the understanding of aging beef.

6. We anticipate that samples will be well received by panelists. However, if we find during the course of the taste panel that samples are unappealing, we will stop the evaluation process.

7. To maintain anonymity of the data collected during this evaluation, all the information you provide will be placed in a locked file with Dr. Murdoch.

8. If you have questions about the taste panel, you can ask the investigator during the evaluation, when the evaluation is complete or at a time you feel is appropriate.

9. Contact information for the University of Idaho faculty member leading this research:
   Dr. Gordon K. Murdoch
   University of Idaho
   Department of Animal and Veterinary Science
   Moscow, ID 83844

10. During the course of this taste panel, you may terminate participation at any time. If you choose to do so, please notify the investigator that you no longer wish to participate.

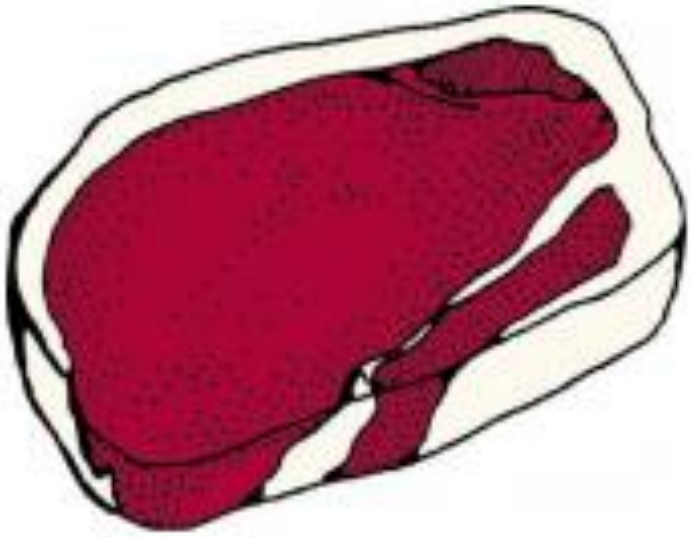
11. If you choose to terminate participation in this evaluation, there will be no penalties associated with your withdrawal.

I have reviewed this consent form and understand and agree to its contents.

Participant Name: _____ Date: _____

Signature: _____ Date of Birth: _____

EVALUATION OF BEEF QUALITY



Panelist #:_____ Date: _____

Age: _____ Gender: _____

Please indicate the number of meals a week in which you consume beef:

0-1 2-4 5-7 8+

Please indicate the form in which you most commonly consume beef:

Ground Roast Steak Other

Thank you for taking the time to participate in this sensory panel

Sensory Panel Questionnaire

Sample ID #:_____

1. **OVERALL ACCEPTABILITY OF SAMPLE:** This is based on your overall acceptability of the sample

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

2. **TENDERNESS:** This is based on your overall opinion of the sample's tenderness

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

3. **JUICINESS:** This is based on your overall opinion of the sample's juiciness

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

4. **FLAVOR:** This is based on your overall opinion of the sample's flavor

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

5. **OFF-FLAVOR:** This is based on your ability to detect an off-flavor of the sample

NO YES

6. **CONSUMER SATISFACTION:** Would you be willing to purchase this product?

NO YES

7. **IF APPLICABLE,** please circle the trait you liked **least** about this product.

Flavor Tenderness Juiciness Texture/Mouth Feel

8. **IF APPLICABLE,** please circle the trait you liked **most** about this product.

Flavor Tenderness Juiciness Texture/Mouth Feel

9. **Overall Comments on Product:**

Appendix F

Solvent Gradient for HPLC Protocol

Appendix F. Solvent gradient for HPLC protocol

Time (min)	% Solvent A[1]	% Solvent B[2]
0	90	10
6	87.5	12.5
38	42	58
71	0	100
79	0	100
84	90	10
94	90	10

[1] Solvent A: 0.14 M Sodium Acetate containing 0.5 mL/L of triethanolamine at pH 6.4 with 5% acetonitrile

[2] Solvent B: 60:40 Acetonitrile and Water

Appendix F: Solvent gradient performed with each HPLC cycle run

Appendix G

TBARS for oxidative rancidity - rapid, wet method

Adapted from Appendix O: TBARS for Oxidative Rancidity (AMSA, 2012)

Principle:

In the presence of thiobarbituric acid (TBA), malonaldehyde and other aldehyde products of lipid oxidation (TBA reactive substances; TBARS) form pink chromogens with maximum absorbance at 532-535 nm. However, in the presence of interfering sugars, a yellow chromagen forms, which can be avoided using the distillation method (Tarladgis, 1960).

Reagents:

1. TBA stock solution - 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25N HCl.

2. Stock solutions (100 mL) are sufficient for 20 individual tests. Stock solution may be stored at room temperature in the dark (foil-wrapped container).

Procedure:

1. Finely chop or mince a portion of the product of interest. Weigh out duplicate 0.25 g samples.

2. Add 1.25 ml TBA stock solution to each sample, giving a dilution factor of 6. Mix well.

3. Heat samples 10 min in boiling water in loosely capped 2.0 ml eppendorf tubes

Caution: tightly capped tubes may burst during heating. Positive samples turn pink during heating.

4. Cool tubes in tap water.

5. Centrifuge at 5,000 × g for 10 min to obtain a clear supernatant.

6. Carefully pipette 200 µl of the supernatant to a 96 well plate. Take care that the solution remains clear.

7. Measure supernatant absorbance at 532 nm against a blank that contains all the reagents minus the meat.

8. Calculate the TBA value expressed as ppm malonaldehyde, using 1.56 × 105 /M/cm as the extinction coefficient of the pink TBA chromogen (Sinnhuber and Yu, 1958), as follows:

TBARS number (mg MDA/kg) = sample A532 × (1 M TBA chromagen/156,000) × [(1mole/L/M] × (0.003 L/0.5 g meat) × (72.07 g MDA/mole MDA) × 1000 mg/g) × (1000 g/kg) or TBARS value (ppm) = sample A532 × 2.77

References:

Buege, J.A. and Aust, S.D. 1978. Microsomal lipid peroxidation. Methods in Enzymology 52:302-304.

Sinnhuber, R.O. and Yu, T.C. 1958. 2-Thiobarbituric acid acid method for the measurement of rancidity in fishery products. II. The quantitative determination of malonaldehyde. Food Technology 12(1):9-12.

Appendix H

Homogenization, Protein Extraction, Derivatization, and HPLC Protocol

Free Histidine, Anserine, and Carnosine Muscle Content – HPLC

Grinding Tissue:

- Cut ~10g sample of thawed muscle tissue, trim edges and exposed surfaces from the strip
- Finely slice the sample into small strips (the smaller, the better)
- Wrap in tinfoil (make pouch easy to re-open) write in Sharpie the sample ID on the foil
- Snap freeze in liquid Nitrogen
- Break apart small bits with hammer
- Place into chilled mortar and pestle (on dry ice, use nitrogen to chill further)
- Grind to fine powder, add nitrogen as needed to keep frozen and prevent sticking to walls of mortar and pestle
- Weigh tissue into labeled 2ml conical bottom tubes at ~250mg each
- Store at -80 C
- REMEMBER TO DIP EVERYTHING IN NITROGEN TO KEEP FROZEN BEFORE COMING INTO CONTACT WITH SAMPLE!
- FREEZE EXTRA TISSUE IN -80 C

Extraction:

- Add bead to frozen tube of 250mg tissue and add 1 mL 0.01 N HCl. Keep samples on ice
- Homogenize at 25Hz for 20 sec 5 times – may need more if tissue isn't all broken down
- Centrifuge chilled samples at 10,000G for 20 min at 4 C
- Filter supernatant through glass wool
    - Place glass wool in Pasteur pipette and filter into clean tube
- Store at -80 C

Deproteinization:

- Methods of Aristov and Toldra (1991)
- Add 50 uL of hydroxyproline (0.325 mg/ml from Sigma) and 750 uL Acetonitrile (HPLC grade) to 250 uL of thawed sample. Stand at room temp for 30 mins
- Centrifuge samples at 10,000 G for 15 min at 4 C
- Immediately derivatize 200 uL of the sample following methods of Bidlingmeyer et al. (1987)

Derivatization:

- Dry samples at 38 C under vacuum and nitrogen in Waters Pico Tag Work Station. Place 200 uL of centrifuged sample in 6 x 50mm glass pyrex tube and cover with square Kim Wipe (DO NOT USE the VWR KNOCK OFF VERSION! IT IS TOO THICK!) and fasten with a rubber band.
- Allow the vacuum pump to run for at least one hour prior to adding samples (Fill the cold finger about ½ with dry ice and fill about 1 inch above the level of dry ice with Methanol).
- Add 20 uL of TEA (methanol-1M sodium acetate-triethanolamine 2:2:1) and dry down. They will look like a transparent gel in the bottom of the tube when drying after this step.
- When drying the samples, the tube must be purged with nitrogen three times for 5 seconds each.
    - Turn on the vacuum knob SLOWLY (or your sample will be sucked up into the Kim Wipe) to between 1-2torr, hold for 5 seconds, close vacuum knob. Open nitrogen knob, purge for 5 seconds, close nitrogen. Repeat this step two more times.
    - Once you have finished purging, open the vacuum slowly until it is held steadily between 1-2torr with no bubbling inside the tubes. You may need to adjust up, turn down, and adjust up again several times to get it to stabilize without sucking up the samples.
    - Come back and re-adjust to 1-2torr in 15-20 minutes
- After dried with TEA, add 20 uL of PITC (methanol-water-triethanolamine-phenyl isothiocyanate 7:1:1:1). Let stand at room temp for 20 mins before drying again. Use the same purging methods described above to dry down the second time. Once dry, samples will be crystallized or gel like, depending on the sample.
- Once dry, add 100 uL of 5mM sodium phosphate with 5% acetonitirle pH 7.6 with glacial acetic acid
- *All solutions for derivatization must be made up fresh each day. Use the PITC in the fume hood!*
- *You can prepare the 5mM sodium phosphate ahead of time.*

HPLC Analysis:

HPLC machine: e2695 separations model equipped with an auto sampler and a Waters 2998 photodiode array detector set to 254nm.

Column: Waters Symmetry C18 reverse 3 x 150 mm (3.5 or 5 uM particle size). Temp is controlled at 40 C ± 1 C with a column heater.

- The method used was adapted from Aristov and Toldra (1991)
- Two eluents were used in the solvent system:

- (A) 0.14 M Sodium Acetate containing 0.5 mL/L of TEA at pH 6.4 adjusted with glacial acetic acid
- (B) 60:40 Acetonitrile Water mixture
- *FILTER THESE TO 0.2 micron before using on the machine! These solutions last about 3 days before they begin to grow microorganisms.*

- The flow rate is set to 0.8 mL/min and the following gradient was performed:

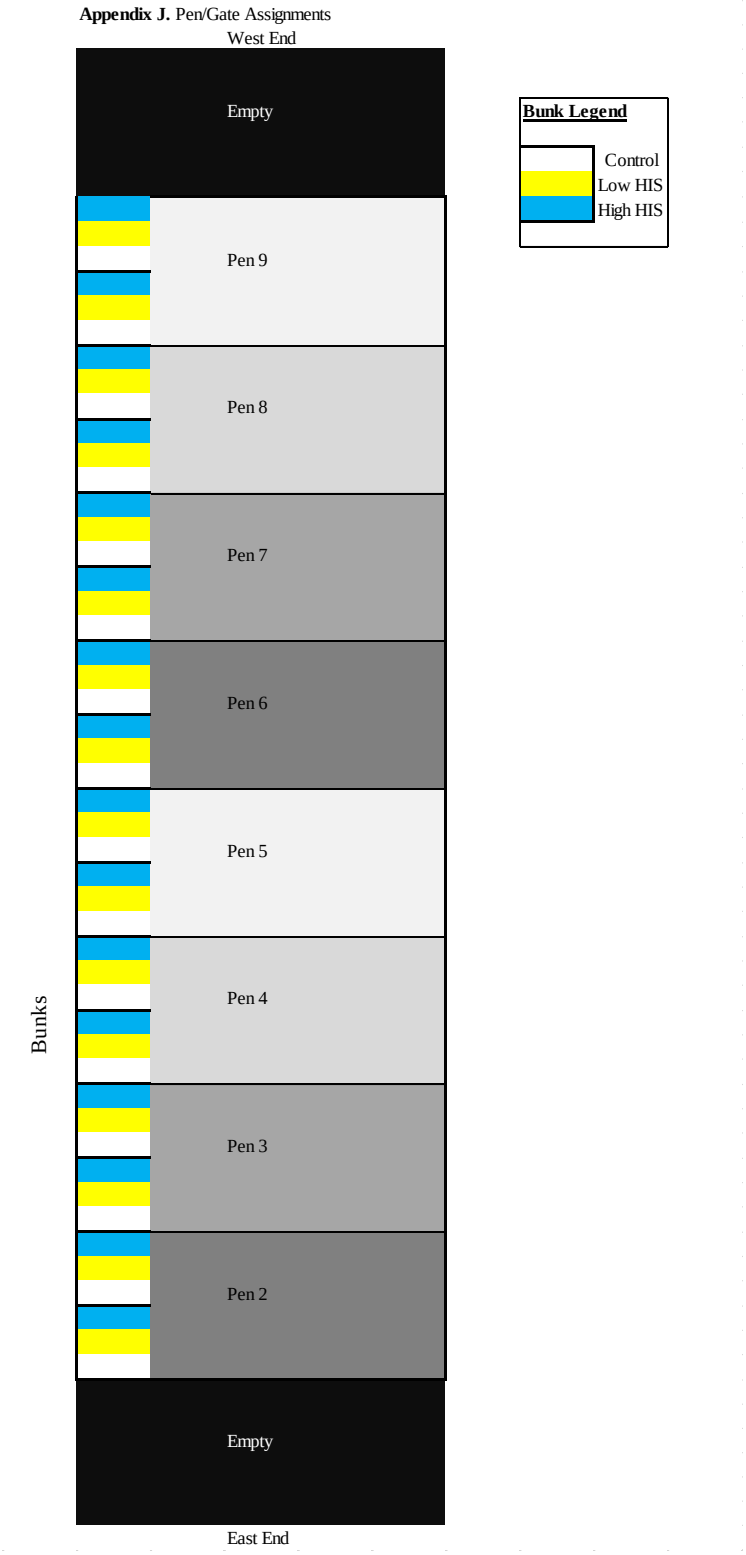
TIME (mins)	***% A***	***%B***
0	*90*	*10*
6	*87.5*	*12.5*
38	*42*	*58*
71	*0*	*100*
79	*0*	*100*
84	*90*	*10*
94	*90*	*10*

- Inject 20 uL of sample into the system
- Four different standards were used: hydroxyproline (reconstituted to 0.325 mg/mL in milipore water), L-anserine (reconstituted to 100mg/mL in 0.5N HCl), L-carnosine (reconstituted to 100 mg/mL in 0.5 N HCl), and L-histidine (reconstituted to 50mg/mL in 0.5 N HCl) (Sigma Aldrich)
  - Hydroxyproline was used as an internal standard. A standard curve was generated at 100%, 75%, 50%, and 25% retention of the hydroxyproline.
  - ANS, HIS, and CARN were run at 0.25 mg/ml, 0.50 mg/ml, 1.00 mg/ml, and 2.00 mg/ml to generate the standard curves.
  - All samples were run in triplicate or duplicated and quantified based on comparison to standard curve. Curves had an R^2 of at least 0.95
  - Free HIS, ANS, and CARN were reported as mg/g wet tissue weight.

  You can make up and aliquot the standards beforehand and store at -80 C for daily use.

Appendix I

Animal Pen/Gate Assignments



Appendix J

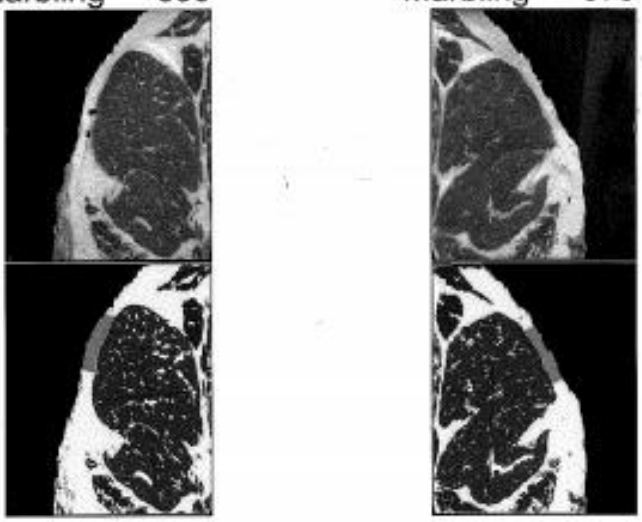
Toppenish Grading System Data

61

VBG 2000 Carcass Report © 2010 Vision For You L.L.C.

Carcass ID	**666033**
HCW	857
Maturity	A
Quality Grade	Prime
Yield Grade	Y2
Marbling	699 / SLAB00
G Program	None
Properties	Angus

Side	1	Side	2
REA	13.83	REA	13.75
Marbling	699	Marbling	579



Appendix K

Animal Care and Use Protocol

University of Idaho
Institutional Animal Care and Use Committee
Annual Protocol Review

Date:	Sunday, August 28, 2016				
To:	Gordon Murdoch				
From:	University of Idaho Institutional Animal Care and Use Committee				
Re:	Protocol 2015-41 Improving colo r and color stability of the top sirloin; EIPRS 7390				
Original Approval:	10/6/201 5	**Annual Expiration:**	10/6/201 6	**3 Year Expiration:**	10/6/201 8

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. This brief renewal application will provide the basis for an annual review for projects that have not changed or may have only minor modifications from year to year. A new protocol must be submitted for new proje cts, major changes in existing protocols, and for all protocols every three years.

1) Please indicate the present status of your project by checking one of the statements below:

____ This project is no longer active, please withdraw.

____ This project is pending/active and there have been no changes in procedures with respect to animal use or personnel.

____ This project is active and there have been changes in the personnel or experimental procedures. (Describe any such changes below.)

2) Please provide a description of any changes in procedures with respect to an imal care and use since your protocol was originally approved. Attach additional pages if necessary.

3) List all changes in personnel involved in your project:

Add	Delete	Name	Department	Email	Training & Experience

Signature: _____ Date: _____

Appendix L: Submitted Conference Abstracts

2017 Pacific Northwest Animal Nutrition Conference

Richland, WA January 17-18

Rumen-protected histidine supplementation; nutrient driven beef improvement

Breann N. Sandberg, Carl W. Hunt, Matthew E. Doumit, Ron Richard and Gordon K. Murdoch
Animal & Veterinary Science
University of Idaho, Moscow 83844-2330

The objective of this study was to determine the effect of supplementation with rumen-protected (RP) histidine in finishing cattle on growth, feed to gain ratio, and carcass traits/product quality. This project tested three levels of daily RP-histidine (control, low dose, and high dose) over a 55-d finishing period and implemented an aggressive 120 d implant (Revalor-XS). Crossbred beef steers were vaccinated, dewormed, blocked by body weight, and randomized into pens of six (eight pens total). The cattle were fed using Calan gates from an average starting BW of 355kg to a finishing LW of 615kg. Cattle were fed twice daily and the morning feed was top-dressed with the RP histidine according to treatment group: control (no RP-histidine), low dose (50g/hd each d), or high dose (100g/hd each d). Each steer received A,D&E injectable vitamin supplementation 80 d prior to initiation of treatments. Individual intakes were recorded, and feed samples and orts were analyzed every 5 d. On d 56, the steers were harvested at a USDA inspected facility, chilled, carcass data recorded at 24 h post-mortem, and fabricated 48 h post-mortem. One *longissimus lumborum* (Striploin: LL) and one *gluteus medius* (Top Sirloin: GM) was obtained from each animal, aged under vacuum (LL: 21 d, GM: 14 d), and cut into 2.54 cm steaks. One steak was used for retail display (9 d) for which subjective color scores as well as objective color scores (using Hunter Mini-Scan: l*, a*, and b*) were recorded on d 0,1,3,5,7, and 9. All samples were analyzed on d 0 and 9 using T-BARs to quantify lipid oxidation. Another steak was weighed and cooked to evaluate purge and cooking loss, and then tenderness was assessed using Warner-Bratzler Shear Force (WBSF). Consumer taste panels were conducted (1 panel for each muscle, 2 total) to determine consumer acceptability.

RP-histidine supplementation improved the lean color, uniformity, and brightness of the product throughout retail display, and decreased surface discoloration, browning, and purge compared with control. Consumers preferred RP treated product over control based upon improved juiciness, increased overall satisfaction and willingness to purchase the product. A trend for increased growth and improved feed to gain was observed in the treated animals. There were no negative effects of RP-treatment in terms of carcass quality (quality grade and yield grade), tenderness, cook loss, texture, flavor, incidence of off-flavor, or pH. Overall, 55 d of pre-harvest RP-histidine treatment positively impacts consumer perception and may optimize product quality and marketability.

2017 ASAS-CSAS Annual Meeting & Trade Show

Baltimore, MD July 8-12

Effects of rumen protected-histidine supplementation dose on finishing beef cattle.

Breann N. Sandberg, Carl W. Hunt, Matthew E. Doumit, Ron Richard and Gordon K. Murdoch
Animal & Veterinary Science
University of Idaho, Moscow 83844-2330

This study examined the effect of dietary rumen-protected histidine (HIS) supplementation in cattle on growth and carcass traits/product quality. Three levels of daily HIS (Balchem corp.) were tested (control: D_O, low dose: D_L, and high dose: D_H) over a 55-d finishing period in 48 cross-bred steers implanted with Revalor®-XS. Cattle were randomly allocated into eight pens of six head each and fed twice daily using Calan gates. Morning feed was top-dressed with the HIS as follows; control (no HIS), D_L (50g/hd/d), or D_H (100g/hd/d). Individual intakes were recorded, and feed and orts were analyzed every 5 d during the feeding period. Steers were harvested, carcass data was recorded, and carcasses fabricated at a USDA inspected facility. One *longissimus lumborum* (LL) and one *gluteus medius* (GM) was acquired from each animal, aged under vacuum (21-d and 14-d respectively) and cut into 2.54 cm steaks. One steak was used for retail display for which subjective (to evaluate visual color components) and objective (l*, a*, and b*) color scores were recorded. Lipid oxidation on d 0 and 9 of retail display were assessed using TBARs. Another steak was used to evaluate cooking loss and tenderness using WBSF. Sensory taste panels were conducted (one panel for each muscle) to determine consumer perception of the product. Muscles were analyzed using HPLC for histidine metabolite (anserine, carnosine) and histidine content.