Effects of Ractopamine Hydrochloride on Color Attributes and Meat Quality in Yearling Heifers Across Days on Feed

by

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Abstract

Recent research with the use of β-agonists has been inconclusive from a meat quality standpoint. Therefore, the objective of this study is to evaluate the inclusion of RAC across 5 DOF in 72 crossbred yearling heifers and how it effects meat quality. Heifers were individually fed twice a day at the Auburn University Beef Cattle Evaluation facility. Heifers were divided based on initial height and weight, and randomly assigned to one of the following DOF groups: 79 (n = 16), 100 (n = 16), 121 (n = 16), 142 (n = 16), or 163 (n = 7). Within these 5 groups, heifers were randomly assigned to a treatment of either 300 $mg \cdot hd^{-1} \cdot d^{-1}$ RAC-HCl (RAC; Elanco Animal Health, Greenfield, IN) for the final 35 d prior to harvest or a control group (CON; 0 $mg \cdot hd^{-1} \cdot d^{-1}$ RAC). Twenty-four hours postmortem, boneless loins were removed from the right side of each animal, vacuum-packaged, and aged for 21 d in the cooler ($2^{o}C$). After 21 d, 7 strip steaks (2.54 cm thick) were cut for sensory analysis, WBSF, and lipid oxidation analysis. The main effect of RAC had no effect ($P > 0.05$) on meat quality. Hot carcass weight was lowest ($P < 0.05$) for DOF group 79 compared to other DOF treatment groups. Backfat thickness and KPH was highest ($P < 0.05$) in DOF groups 121 and 142. Intermediate fiber area was highest ($P < 0.05$) for DOF group 121. The percentage of red fibers was highest ($P < 0.05$) for DOF group 121. Compared to all other treatments, DOF group 163 RAC steaks were less tender for initial and sustained tenderness. RAC supplementation had no effect on carcass characteristics and there were no deleterious effects on meat quality factors by the addition of RAC into the diet.

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I. LITERATURE REVIEW

Introduction

In today's market, increasing input costs for producers have directed research efforts towards finding more cost-effective production options. Improved methods for decreasing the feed required per unit of gain in live animals are essential to alleviate input costs wherever possible. In order to decrease the amount of feed consumed in an animal's life, certain repartitioning agents, known as β-agonists, have been developed to increase average daily gain in the animal, increase lean muscle accretion, and decrease fat accretion (Mersmann, 1998). Because of this increase in ADG, meat animals reach desired harvest weights in a shorter amount of time. At the same time, a decrease in subcutaneous fat and increase in lean muscle should result in lower USDA yield grades resulting in an overall increase in profit. Data published in the early 1980's reported data published by the Cyanamid company which revealed that oral administration of clenbuterol, a β-adrenergic agonist, increased muscle mass and decreased fat mass in growing cattle, pigs, chickens, and sheep (Ricks et al., 1984). In more recent years, β-adrenergic agonists such as Ractopamine hydrochloride (RAC), which was the first β-agonist approved by the FDA in 2003 for use in cattle, have been implemented into the production system. These β-agonists tend to increase lean muscle, through muscle hypertrophy, and decrease tenderness, possibly due to increased levels of proteolytic inhibitors and decreased protein degradation. This review will cover the published literature related to the relationship between β-agonists and their effects on skeletal muscle growth, tenderness, shelf-life stability, and consumer acceptance.

Muscle Structure

There are two main protein components which make up the muscle, consisting of the muscle and connective tissue. Within these two compartments are three classes of proteins as described by Goll et al. (2008). First, the stroma proteins make up approximately 10-15% of total protein and can be classified as those that make up the connective tissue system, with the major contributor being the fibrous protein, collagen. The sarcoplasmic proteins constitute approximately 30-35% of the total protein in the muscle, are soluble in water, and are typically enzymes involved in the metabolic pathway, creatine kinase, and myoglobin (Tornberg, 2005). The majority of muscle protein is represented by myofibrillar proteins (55-60%), which includes the major muscle proteins actin and myosin (Goll et al., 2008). The myofibrillar proteins are responsible for the contractile properties of the muscle and therefore can have adverse effects on meat quality depending on the mechanisms in the muscle. The myofibrillar proteins are the majority of the total protein content in the muscle and are located within the myofibril. This group is composed of a vast number of proteins, which are all involved in processes related to muscle structure, and more specifically sarcomere structure. The sarcomere is the basic contractile unit in the myofibril. The muscle fiber is made up of many proteins, which serve as both regulatory and structural proteins. These proteins range from those found in the Z-disk of the sarcomere to those that are associated with the structure of the myofiber. Within the sarcomere are two myofibrillar proteins known as myosin and actin, which are components of the thick and thin filaments, respectively.

Aside from the proteins in the lean muscle, connective tissue is also a large component within the animal. Collagen is the main protein of connective tissue in muscle. It is the most abundant mammalian protein, providing a network of fibers in all tissues (McCormick, 1999).

Presently, 19 known forms of collagen have been identified, which hold roles in a variety of biological systems (McCormick, 1999). The structure of various collagen forms differ from mesh-like networks of Type IV collagen to long, filamentous fibers typical of Types I, II, and III. The basic structure of connective tissue in muscle includes three compartments known as the epimysium, perimysium, and endomysium.

The epimysium surrounds an individual muscle and is continuous with the tendon joining other muscles or bones in the epimysium (McCormick, 1999). Due to its support role, the epimysium is often very thick, and is resistant to both heat and force. Although very tough, it generally does not affect meat quality because it is typically separated from meat in order to avoid decreases in quality. The perimysium is responsible for covering muscle bundles, or fascicles, and is predominantly composed of collagen types I & III (Purslow, 2005). Along with surrounding muscle bundles, the perimysium also contains intramuscular lipid deposits and vasculature. There are large or primary fascicles and small or secondary fascicles, and therefore primary and secondary perimysial layers separate them (Purslow, 2005). Finally, the endomysium is the innermost constituent of the collagen compartments, which encompasses the individual muscle fibers and overlays the basement membrane. Although, the intramuscular connective tissue is a combination of both the peri- and endomysiums, the perimysium constitutes about 90% of the tissue (McCormick, 1999). Therefore, the perimysium is a major determinant of meat quality and more specifically, meat tenderness (Purslow, 1999). One way that the perimysium affects meat quality is that its thickness increases as an animal ages (Brooks and Savell, 2004)

Skeletal Muscle Growth

Current research is focused on improving methods for producing more efficient, lean animals. These characteristics can be achieved by a number of factors associated with muscle growth including an increase in muscle fiber number, fiber size, and a shift from small, oxidative slow-twitch fibers to large, glycolytic fast-twitch fibers (Wegner et al., 2000). There are also studies suggesting that fiber type and size has an impact on overall eating quality, including tenderness (Crouse et al., 1991; Maltin et al., 1998), but this relationship still remains unclear. Other studies, including Whipple et al. (1990) and Vestergaard et al. (2000) concluded that there was no correlation between fiber type and tenderness.

Muscle growth is a result of the increase in number of muscle fibers (hyperplasia) and growth of the individual fiber (hypertrophy). Muscle fibers are formed during the embryonic and fetal stages of life prior to birth. The number of muscle fibers is thought to be fixed at birth in most mammals (Andersen, 2005). Therefore, postnatal growth is the increase in cross-sectional area and the addition of sarcomeres contributing to the length of the muscle fibers. Muscle development of an animal is a combination of both hyperplasia and hypertrophy both in prenatal and postnatal development. However, hypertrophy is determined by both intrinsic and extrinsic factors, and occurs as the animal ages (Koohmaraie et al., 2002). Intrinsic factors can be breed type and sex of the animal, whereas extrinsic factors may include feeding regime and environmental conditions that the animal is exposed to. The amount of muscle growth is determined by the amount of protein synthesis as well as the protein degradation (Koohmaraie et al., 2002). The difference in these two factors is the determination of overall protein accretion. This process can be affected by three possible scenarios; a) increased protein synthesis and decreased protein degradation, b) protein synthesis in amounts greater than the amount of protein

degradation, c) protein synthesis in amounts lesser than in protein degradation (Koohmaraie et al., 2002). All of these factors can have both positive and negative results on meat tenderness.

Muscle fiber types are characterized by their metabolic capabilities, either oxidative or glycolytic metabolism (Klont et al., 1998). The type I fibers, also known as β-red fibers, are slow-twitch and exhibit oxidative metabolism. These fibers appear red after staining due to the number of mitochondria in the cell. Type IIa, or α-red fibers, are slow twitch and can metabolize both anaerobically or aerobically. Finally, the type IIb, or α-white fibers, are those that are fast-twitch, glycolytic fiber types.

As previously stated, there are two types of α-fibers, which are either intermediate or white fiber types. Many studies conclude there is a transformation postnatally which determines whether a fiber will be intermediate or white (Ashmore, 1974). Following birth, the transformation of α fibers into either α-red or α-white is caused by the function of the muscle. Results of Ashmore (1974) suggested that muscles that are relatively dormant show a transformation from α-red to α-white, as well as a rapid growth in diameter. In contrast to α fibers, β fibers grow at a much slower rate and do not undergo a transformation. This theory also supports the idea that β fibers are located internally in the fiber bundles of the muscle, whereas α fibers are mostly seen on the peripheral areas of the muscle (Ashmore et al., 1972).

Ashmore, Tompkins, and Doerr (1972) conducted an experiment using ovine, porcine, and bovine muscles to determine whether the transformation of α fibers occurred similarly in these species as it had in a previous experiment by a group of chicks. The current study again showed that the muscles from ovine, porcine, and bovine did undergo transformation of α fibers and the transformation appeared to be primarily concerned with energy-producing enzymes. As mitochondrial density decreased, glycogen phosphorylase increased. Glycogen phosphorylase is

indicative of glycogen enzymes in general and is an indicator of anaerobic or glycolytic metabolic processes.

Examining the typical growth curve is important when conducting studies which affect the animal's nutrition, and thus, affecting animal growth. As changes are made in the diet, whether it is through the use of β-agonists or growth promotants, it is important to acknowledge they will undoubtedly affect the animal's growth, and can cause a shift in fiber types. Vestergaard et al. (1994) stated that β-agonists increase the percentage of type IIA fibers at the expense of type I fibers.

Postmortem Degradation and Meat Tenderness

Meat tenderization is achieved through a number of processes and can be affected by many factors. The end product of meat tenderization is the breakdown of myofibrillar proteins and the ultimate degradation of the Z-line in the sarcomere (Koohmaraie, 2002). In order for these processes to occur there is an enzyme system which is involved in protein degradation known as the calpain system (Koohmaraie, 2002). Calpains are calcium-dependent enzymes that bind to calcium, allowing the enzymes to breakdown proteins. The two basic calpain types are known as μ- and m-calpain, which differ in the amount of calcium required to activate the enzyme. Therefore, μ-calpain is activated by very low levels of calcium, while m-calpain requires higher levels. Because of their role in the breakdown of proteins, the calpains are thought to be the rate-limiting step in postmortem proteolysis (Goll et al., 2008). The known inhibitor of the calpains is calpastatin. Many studies have attributed decreased tenderness to increased levels of calpastatin in meat products because of their inhibition of the calpain system in proteolysis (Koohmaraie et al., 1991; Morgan et al., 1993)

Postmortem proteolysis results in degradation of the structural components of the sarcomere, and more specifically the Z-disk (Koohmaraie, 2002). The Z-disk is comprised of many proteins which enhance the structural integrity of the sarcomere. In order for proteolysis to degrade the proteins of the Z-disk, the calcium-dependent calpains must be activated. Several studies have shown degradation of the Z-disk begins within the first 24-72 h postmortem, and degradation continues for 14-21 d. Wheeler and Koohmaraie (1994), illustrated there is a large increase in shear value from 1-12 h (5.07 kg to 8.24 kg) postmortem. Following this increase in shear force values, there was a steady decline between 12 and 72 h postmortem, in which shear values decreased from 8.24 kg to 4.36 kg. Following 72 h., shear force decreased at a decreasing rate. By d 14 postmortem, shear force values had decreased to an average of 3.10 kg.

Along with myofibrillar tenderness, connective tissue tenderness is one of the major determinants in overall meat tenderness and accounts for "background tenderness" in the product (Bailey, 1972). There are many factors affecting the amount of collagen, as well as collagens affect on meat tenderness. These factors can range from live animal factors, such as age, to factors associated with meat cookery (Purslow, 2005).

Collagen has been shown to decrease the tenderness of meat because of the formation of cross-links in the muscle (Nishimura et al., 1995). This cross-link formation is often a result of animal age. Many studies have shown the total collagen content increases as an animal ages, which results in the replacement of reducible crosslinks with mature forms (hydroxyproline crosslinks), because of a decrease in the rate of collagen turnover (McCormick, 1999; Harper et al., 1999; Purslow, 2005). These crosslinks are assembled by formation of covalent bonds, which replace hydrogen bonds. As total collagen content increases, there is also a decrease in soluble collagen in muscles from older animals, which results in higher amounts of insoluble

collagen. Therefore, the muscle may suffer from tenderness issues because cooking temperatures will not breakdown insoluble collagen.

With the understanding that animal age increases the number of mature crosslinks in the muscle, research efforts have focused to increase collagen turnover due to nutrition, resulting in newly synthesized collagen. In a study conducted by Boleman et al. (1996), mature cows were placed in feedlot conditions for 0, 28, 56, and 84 d prior to slaughter. Through the use of a high-concentrate diet, this study showed newly synthesized heat-soluble collagen tended to increase in the muscle. Another study by Aberle et al. (1981) showed an intensive preslaughter feeding regimen for 70 d, increased the percentage of soluble collagen as a result of increased rate of protein synthesis.

Although age plays a major role in the formation of collagen, there are also other factors associated with increases in total collagen content. These factors include muscle and collagen type within the muscle. Locomotive muscles, such as the *semimembranosus* in the round, have shown to contain more collagen than support muscles such as the *longissimus* (Brooks and Savell, 2004). As previously mentioned, soluble collagen content within the muscle is susceptible to breakdown with heat and therefore does not cause tenderness issues unlike insoluble collagen does.

β-adrenergic agonists

Beta-agonists have been utilized for many years in research in beef cattle, chicken, sheep, and swine. Cunningham (1965) was the first to present data showing that growth could be changed by administering agents such as caffeine, theophylline, nicotine, and epinephrine. It was not until the 1980's that data was published which indicated a model of growth for animals fed the β-agonist, clenbuterol, which increased muscle and decreased fat mass (Ricks et al., 1984). The β-

agonists are also referred to as repartitioning agents because they alter muscle tissue without effecting bone or organ mass (Beerman, 2002). Various β-agonists have different effects on animals due to factors such as species, duration of agonist feeding, dosage levels and sex effect. Williams (1989) suggested that the lack of response in very young nursing lambs and in other species to β-agonist treatment is due to an insufficient number of receptors or that growth is already at its peak rate in very young muscle fibers.

Ractopamine hydrochloride (RAC; Elanco Animal Health; Greenfield, IN) was the first β-adrenergic agonist approved by the US Food and Drug Administration (FDA) for use in cattle finishing diets to increase rate of weight gain, improve feed efficiency and increase carcass leanness when fed the last 28 to 42 d (Schroeder et al., 2004a), prior to harvest. Ractopamine, which is a part of the phenethanolamine class of compounds, is responsible for binding to β-adrenergic receptors in order to elicit the β-agonist response in livestock species. For a β-agonist to have biological activity, it must have a substituted six-membered aromatic ring, hydroxyl group bonded to the β-carbon in the R configuration, positively charged nitrogen in the ethylamine side chain, and bulky substituent on the aliphatic nitrogen to allow specificity for the β-receptor (Weiner, 1980; Smith, 1998). Slight modification to the structure of β-agonists causes differences in biological activity. More specifically, structural changes seen on the aromatic ring groups and the bulky R-groups off of the aliphatic nitrogen, account for differential responses elicited by β-agonists (Mersmann et al., 1998). It is also known that the addition of halogens increase the lipophilicity of the aromatic portion of the β-agonist opposed to the hydroxylated aromatic ring (Smith, 1998). The structure of phenethanolamines is similar to that of catecholamines, which include the natural adrenergic neurotransmitters epinephrine and

norepinephrine that only differ in the bulky group on the aliphatic nitrogen. Therefore the mechanism for which a β-receptor binds to the agonist is similar.

Mersmann (1998) reviewed the mechanism associated with the action of the β-adrenergic agonist and receptor relationship in order to stimulate a response of epinephrine and norepinephrine, which are known physiological β-adrenergic receptor agonists. This mechanism begins with the activation of the G_s protein. This is achieved by the binding of the organic molecule, the β-adrenergic agonist, to a β-adrenergic receptor. These receptors are present in most mammalian cells, but the distribution of subtypes is dependent on tissue location and species (Mersmann, 1998). Once the G_s protein is activated by the agonist-receptor complex, the α-subunit of the G_s protein activates the enzyme adenylyl cyclase. This enzyme is responsible for the production of cyclic adenosine monophosphate (cAMP). This intracellular signaling molecule can then bind to the regulatory subunit of protein kinase A (PKA) to release the catalytic subunit to phosphorylate intracellular proteins. Once PKA is activated, a number of activities can be present. For example, many proteins can be activated when PKA is phosphorylated including hormone sensitive lipase, which is the rate-limiting enzyme for lipolysis (Mersmann, 1998). Also, enzymes such as acetyl-CoA carboxylase, which is the rate-limiting enzyme for lipogenesis, are also inactivated due to phosphorylation. Finally, PKA can have an impact on gene expression by phosphorylating transcription factors. For example, gene expression can be activated or inactivated by the phosporylation of cAMP response element binding (CREB) protein (Mersmann, 1998). Due to the activation of receptors which have effects on enzymes involved in both lipogenesis and lipolysis, the final amount of fat deposited in the animal is affected by β-agonists.

Dosage, length of supplementation, and sex effects

External factors such as dose, length of treatment, and sex effects, are also associated with the magnitude of the β-agonist response (Moody et al., 2000). Sainz et al. (1993) showed pigs fed 20 mg/kg RAC for 6 wk had an increase in average daily gain from week 0-3, but showed no improvement in the final 3 wk prior to slaughter. In a study conducted by Chikhou et al. (1993), steers were fed cimaterol from 4 wk of age until slaughter. These results concluded there was little effect on growth rate and gain efficiency in comparison to trials that fed β-agonists for a shorter amount of time. In contrast, Beerman et al. (1986, 1987), showed that lambs fed cimaterol for approximately 2 mo had a 25-30% increase in the weights of several muscles compared to those lambs on the control diet. Based on all of these studies, it is evident that β-agonists can have differing effects based on many factors.

When evaluating the response of RAC in heifers compared to steers, studies have shown a decreased response in certain carcass characteristics. Schroeder et al. (2004b) reported no change in dressing percentage for heifers, whereas dressing percentage of steers in the same study had increased values when fed 200 or 300 $mg \cdot hd^{-1} \cdot d^{-1}$ RAC. Additionally, ribeye area and carcass maturity were only improved in heifers fed the maximum dosage level approved by the FDA (300 $mg \cdot hd^{-1} \cdot d^{-1}$).

Carcass characteristics and days on feed

In respect to cattle, conflicting results for carcass characteristics in steers and heifers finished on RAC are found in the literature. Laudert et al. (2004) showed that yearling beef steers had increased average daily gain, improved feed efficiency, increased carcass weights, and limited effects on USDA yield and quality grades. In steers finished for the final 28 and 42 d on 0-300 $mg \cdot hd^{-1} \cdot d^{-1}$ RAC, Schroeder et al. (2004a) found heavier hot carcass weights, increased

ribeye area, increased dressing percent for 200 and 300 mg·hd^{-1}·d^{-1}fed steers, and higher yield grade in 300 mg·hd^{-1}·d^{-1}group. The same trial was conducted using heifers in which differences were found only in hot carcass weight and ribeye area. These results showed that hot carcass weights was increased in only the 200 and 300 mg·hd^{-1}·d^{-1}RAC groups and ribeye area was increased in heifers fed 300 mg·hd^{-1}·d^{-1} RAC. A similar trial on heifers concluded that there were no differences in hot carcass weight or ribeye area (Quinn et al., 2008). These results contrast the results by Schroeder et al. (2004a) as well as Gruber et al. (2007) in which these two studies found no differences in hot carcass weight and dressing percentage on heifers finished on 200 mg·hd^{-1}·d^{-1} RAC in the final 28 d of the finishing phase.

Beta-agonist action in beef cattle is also affected by the number of days on feed. Previously, studies such as Winterholler et al. (2007) showed that yearling steers fed RAC increased hot carcass weight, dressing percentage, and ribeye area when time on feed increased. Marbling score and USDA yield grade both improved. These results were comparable with May et al. (1992) as well as Van Koevering et al. (1995) which concluded that an increase in days on feed would increase yield grade and subcutaneous fat cover. All of these projects show that increased days on feed, with or without RAC supplementation, reach a harvest optimum, which is consistent with the animal's growth curve.

One negative effect of increasing days on feed, is that the maturity of the animal increases. As previously stated, animal age results in the development of greater amounts of insoluble collagen, resulting in a less tender product. Shackelford et al. (1995) reported differences in overall tenderness and ease of fragmentation between yearling heifers and 24 – month-old cows. As early as 1970, Zinn et al. (1970) concluded that there was a significant

interaction between days on feed and tenderness. Cattle fed 180 days were significantly tougher than cattle fed for 120 to 150 d.

Beta-agonists and muscle growth

The β-agonists have shown to be the result of true muscle hypertrophy in contrast to other types of muscle growth such as compensatory growth (Yang and McElligot, 1989). In response to muscle hypertrophy, cross-sectional area of type II fibers have consistently been reported to increase in both rats and lambs due to β-agonist treatment (Maltin et al., 1986; Zeman et al., 1988). Along with muscle hypertrophy, β-agonists have also shown to increase the percentage of type IIA fibers while decreasing type I fibers (Vestergaard et al., 1994; Rajab et al., 2000; Bricout et al., 2004). However, anabolic agents have no effect on the distribution of type I or type IIA fibers (Ono et al., 1996; Fritsche et al., 2000). Maltin et al. (1986) conducted a study of rats fed clenbuterol for 4 or 21 d. These researchers found an increase in cross-sectional area of slow-twitch, oxidative fibers (type I), however there was no reported change in the composition of fibers. There was also an increased response to clenbuterol after four days opposed to 21 d, and there was a decrease in type IIa fibers and an increase in Type IIb fibers. In contrast, Zeman et al. (1988) conducted another experiment in which rats were fed clenbuterol. These results showed hypertrophy in fast-twitch muscles, but not in slow-twitch histochemically identified muscles. There was also a reported increase in fast-twitch to slow-twitch fiber ratio. However, in a study conducted on lambs treated for 8 wk, there was no muscle hypertrophy in Type I fibers and no change in fiber type proportion (Kim et al., 1987). In more recent years, Beerman (2002) reviewed the increase in hypertrophy of type II fibers and accounted for the increase in muscle mass without any quantitative change in muscle length.

Warner-Bratzler shear force and sensory characteristics of cattle finished on β-agonists

In the meat industry, consumer acceptance is one of the most influential aspects of change. A certain level of consumer acceptability must be obtained in order for a product to have potential profits. Recent research has found tenderness to be the most important factor associated with consumers continuing to purchase a product aside from visual parameters (Savell et al., 1987, 1989; Smith et al., 1987; Miller et al., 1995).

The American Meat Science Association (AMSA, 1995) published recommendations for using both sensory panels and shear force measurements in different species. Sensory panel evaluation serves as a measurement in order to rate meat products based on certain attributes such as tenderness and juiciness. Panelists are trained in order to identify certain characteristics within the meat product in order to determine if that product is more or less palatable.

An objective measurement for tenderness typically used is Warner-Bratzler shear force (WBSF), which measures the amount of force needed to shear through a meat sample. Through the years, an accepted scale has been adopted in order to correlate WBSF numbers with acceptable tenderness levels. Through the work of many studies (Miller et al., 1995; Huffman et al., 1996; Boleman et al., 1997; Shackelford et al., 1991 1997, 1999; Wheeler et al., 1999; and Miller et al., 2001), definitive thresholds have been established in order to classify meat tenderness based on a mixture of steaks from the rib, loin, chuck, and round cooked to a medium degree of doneness. These thresholds can be categorized as the following: 1) Tender- shear force values between 6.6-9.0 lbs (2.99-4.08 kg) or less, 2) Acceptable or Intermediate- shear force values ranging from 9.0-13.0 lb. (4.08-5.90 kg), 3) Unacceptable or tough- shear force values 13 lb. or greater (>5.90 kg; Schroeder et al., 2004b).

With the use of β-agonists in cattle finishing diets, consumer approval has been studied in order to document sensory properties in meat products. Tenderness has been of most concern with the use of β-agonists not only due to its importance to consumers, but also because of initial concerns of decreased tenderness associated with the use of β-agonists. Some researchers have concluded a decrease in tenderness could be a result of increased protein synthesis (Maltin et al., 1987) or decreased protein degradation (Reeds et al., 1986). Others have found a decrease in proteolytic enzymes and an increase in the amount of proteolytic inhibitors (Kretchmar et al., 1990). This is mainly an increase in calpastatin, which inhibits the ability of the proteolytic enzymes to undergo the tenderization process in meat postmortem. Kretchmar et al. (1990) used lambs and concluded β-agonist treatment results in a decrease in proteolytic capacity in the muscle.

Schroeder et al. (2004b), examined sensory properties of cattle finished on 100, 200, or 300 $mg \cdot hd^{-1} \cdot d^{-1}$ RAC compared to the cattle on a control diet with no RAC supplementation. The authors found that no differences in sensory characteristics, including initial and sustained tenderness, for cattle finished with 100 or 200 $mg \cdot hd^{-1} \cdot d^{-1}$RAC. However, when the dose of RAC was increased to 300 $mg \cdot hd^{-1} \cdot d^{-1}$, differences were seen in initial and sustained tenderness, which included a decrease in tenderness. No differences for shear force were seen for cattle treated with 100 and 200 $mg \cdot hd^{-1} \cdot d^{-1}$ (1.58 and 1.65 kg), but when the dosage was increased to 300 $mg \cdot hd^{-1} \cdot d^{-1}$, shear force measurements increased to 1.79 kg, which still falls within the tender threshold. According to this study, no differences were seen in tenderness measurements for cattle supplemented with RAC except for the 300 $mg \cdot hd^{-1} \cdot d^{-1}$RAC treatment.

In a more recent article comparing the three β-agonists zilpaterol, clenbuterol, and RAC, Strydom et al. (2009) concluded that both zilpaterol and clenbuterol had increased shear force

values when compared to control diets at 2, 7, and 14 d postmortem. However, RAC was also used as a treatment in this study, and shear force values showed no differences at the specified days postmortem compared to control. This study did not perform a sensory panel to obtain subjectiv measurements.

Quinn et al. (2008) completed a trial using 200 $mg \cdot hd^{-1} \cdot d^{-1}$ Optaflexx on heifers. These results concluded that once again, shear force values for cattle treated with RAC were not different from those cattle fed the control diet. Finally, a project conducted by Avendano-Reyes et al. (2006) finished steers for the final 33 d on 60 $mg \cdot hd^{-1} \cdot d^{-1}$ zilpaterol (Zilmax®) and 300 $mg \cdot hd^{-1} \cdot d^{-1}$ RAC (Optaflexx®). These results found that both β-agonists had greater shear force values (zilpaterol: 5.06 vs. 4.34 kg; RAC: 4.75 vs. 4.31 kg) in comparison with control steers. Once again, it is important to note that this study fed RAC at the 300 mg level, which showed increased shear force values in this study as well as in the study conducted by Schroeder et al. (2004b). According to the literature mentioned above, studies in which RAC levels exceed 200 $mg \cdot hd^{-1} \cdot d^{-1}$ have shown increased shear force values in beef cattle. Zilpaterol has shown increased shear force values in all studies reviewed.

Meat Color

Meat color is the most important factor associated with a consumer purchasing a product, because any discoloration suggests to consumers that a product is not fresh (Mancini et al., 2005). Nearly 15% of retail beef results is sold at a discounted price due to the discoloration of the meat surface, which gives rise to an annual revenue loss of over $1 billion (Smith et al., 2000). Due to the potential revenue loss from discoloration, it is important for researchers to understand the mechanism and factors associated with meat color.

Myoglobin, which is the primary protein responsible for meat color is a water-soluble protein containing eight helices (A-H) linked by short non-helical sections (Mancini et al., 2005). It consists of a protein portion known as globin and a non-protein portion, which is a heme ring. Within the heme ring is a centrally located iron which contains 6 coordination sites that bond to different elements. Iron is an element that is found in two chemical states; as the ferric (oxidized) and ferrous (reduced) forms. A determination of the opportunity for oxygen to bind myoglobin is dependent on the state that the iron atom is in. Four of the 6 coordination sites bind the iron atom to the heme ring, the 5^{th} site binds to the amino acid (typically histidine), and the 6^{th} and final site is open (ligand) to bind oxygen when present. The opportunity for the ligand to bind oxygen results in different states of myoglobin color seen in meat. Three important color pigments that the myoglobin can become are deoxymyoglobin, oxymyoglobin, and metmyoglobin.

Deoxymyoglobin is the result of the iron atom of the heme ring being in the ferrous (reduced) form (Fe^{2+}; Mancini, 2005). As a result of the iron being reduced, there is no ligand present at the sixth coordination site. A purple-red meat color is the result of deoxymyoglobin, which is typical of a meat product following initial cutting. For deoxymyoglobin to maintain its color, there must be a very low oxygen tension (<1.4 mm Hg; Brooks, 1935). As meat is exposed to oxygenated environments, the color begins to take on a bright, red color. This bright, red color is known as oxymyoglobin and is the color consumer's associate with fresh meat. During oxygenation, there is no change in the valence of iron, but diatomic oxygen binds to the sixth coordination site. The stability of oxymyoglobin is dependent on many factors including pH, temperature, and oxygen competition by other respiratory processes (Mancini, 2005). Metmyoglobin is the result of a loss of electron of the iron atom, changing it from the ferrous

(Fe^{2+}) state to the ferric state (Fe^{3+}), also known as oxidation (Livingston & Brown, 1982; Wallace et al., 1982). Oxidation results in a brown color in the meat and is typical of meat that has been overwrapped in oxygen permeable film, and placed in retail display for several days. There are numerous factors associated with the formation of metmyoglobin, which include pH, oxygen partial pressure, temperature, light, and reducing activity of the meat (Mancini, 2005).

Although there are subjective methods for measuring meat color, objective measurements such as L*, a*, and b* are utilized. These values are representative of the color range of black to white (L*), red to blue (a*), and yellow to green (b*), which is referred to as instrumental color (Mancini, 2005). An increase in numerical value for each of the color ranges results in an increase of lightness, redness, and yellowness (Mancini, 2005). For purposes of this project, it is important to review the affects of RAC supplementation on color stability. In a study conducted by Gonzalez et al. (2009), RAC was fed for the final 28 d of a feeding trial on beef steers. The L*, a*, and b* color measurements were observed for six muscles. In this study, RAC supplementation had no affects on color stability during retail display. In addition, a study by Quinn et al. (2008), reported that feeding 200 $mg \cdot hd^{-1} \cdot d^{-1}$ RAC for the final 28 d of the finishing period, showed no differences for L*, a*, and b* in heifers.

Lipid oxidation

Although meat production has shifted to an increase in lean and a decrease in fat composition, lipids still provide important meat quality components such as flavor (Wood et al., 2004). The lipid in meat is comprised of a large number of fatty acid residues. A fatty acid is essentially made up of a long carbon chain with a carboxylic acid group on the end. Saturated fatty acids are those that contain no double bonds in the carbon chain, while chains containing one or more double bonds make up mono- and polyunsaturated fatty acids, respectively.

One of the major factors associated with meat deterioration is lipid oxidation (Fernandez et al., 1996). Meat quality is affected by lipid oxidation because it is a contributor to the development of off-flavors such as rancidity in meat products. Lipid oxidation in meat products is caused by the initiation phase in which a fatty acid is involved in a hydrolysis reaction resulting in the formation of a free radical on a carbon within a lipid (Morrissey et al., 1998). Following the formation of the radical, a triplet oxygen can react with the free radical forming peroxy-radicals (Morrissey et al., 1998). These peroxy-radicals are referred to as primary oxidation products and are not stable for a long period of time. At this point, another singlet oxygen (typically from another fatty acid) can continue to react with the peroxy-radical forming secondary oxidation products (Morrissey et al., 1998). Secondary oxidation products are typically ketones and aldehydes. Auto-oxidation continues to occur, resulting in the formation of off-flavors and the production of malonaldehyde. Malonaldehyde formation is analyzed in order to quantify the amount of lipid oxidation taking place in the product. Malonaldehyde measurements are typically analyzed using a thiobarbituric acid reactive substance (TBARS) assay. The TBA can react with the aldehyde groups that are formed in the secondary oxidation products, which gives the ability to measure the amount of oxidation taking place in the muscle.

There is little research known to evaluate the effect of RAC on lipid stability in beef. A recent study conducted by Apple et al. (2008) reported that pigs fed RAC prior to slaughter and placed in retail display for 5 d showed no effect on lipid stability. This study suggests that RAC would have no effect on the lipid stability of beef steaks as well.

Summary

The effects of β-agonists on pigs, beef, and lambs have been thoroughly researched over the last twenty years. Due to these research efforts there is extensive evidence that β-agonists contribute

to increased average daily gain in the animal as well as serving as a repartitioning agent from fat to lean accretion in the muscle. In addition, it is also concluded that the increase in muscle size is due to muscle hypertrophy and is seen particularly in the Type II muscle fiber types. Past research has also attributed the optimum dosage of a repartitioning agent, such as RAC, will produce desirable production traits and meat composition. Based on the literature reviewed, appropriate dosage of RAC in cattle can alleviate input cost and result in increased value due to increased lean weight opposed to fat. Although there are many aspects of research in the area of β-agonists that is agreed upon by researchers, there are also some areas, such as factors associated with tenderness, that still remain inconclusive. Therefore, the objective of this experiment will be to determine the effect that RAC hydrochloride has on muscle fiber types, color attributes, and meat quality in yearling heifers across five days on feed groups.

II. MATERIALS AND METHODS

Animals

All experimental procedures performed at Auburn University were approved by the Auburn University Institutional Animal Care and Use Committee (AU-IACUC, PRN 2007-1273). Seventy-two commercial crossbred yearling heifers were purchased from seven Alabama beef producers for this study. For 66 d prior to the experiment, heifers grazed on summer perennial pasture mixture (bermudagrass: *Cynodon dactyloncl* and bahiagrass: *Papspalum notatum Flusge*) and were fed soyhull pellets (3.2 $kg \cdot hd^{-1} \cdot d^{-1}$). Following this feeding period, heifers were transported to the Auburn University Beef Cattle Evaluation facility (AUBCE) where the remainder of the experiment was completed.

Animals were divided into one of five DOF groups based on initial weight and height. Days on feed groups were: 79 (n = 16), 100 (n = 16), 121 (n = 16), 142 (n = 16), or 163 (n = 7). Within DOF groups, randomly selected animals received 300 $mg \cdot hd^{-1} \cdot d^{-1}$ Ractopamine HCl (RAC; Elanco Animal Health, Greenfield, IN) or 0 $mg \cdot hd^{-1} \cdot d^{-1}$ RAC (CON) for the final 35 d prior to harvest.

Heifers underwent a 21 d warm-up period prior to experimental feeding. At this time, animals were acclimated to individual Calan gates® for feed intake measurement. During the warm-up period, heifers received 2% BW of a grain diet (Table 1) and hay mixture (bermudagrass and bahiagrass). In addition, 0.5 $mg \cdot hd^{-1} \cdot d^{-1}$ of melengestrol acetate (MGA) was added to the diet to suppress estrus during the feeding period. Following the warm-up period, heifers had ad-libitum access to the diet fed twice a day for the remainder of the experiment.

Three heifers were removed from the growth analysis (DOF group 79, 142, 163) because they were not trainable to the Calan gates®. In addition, one heifer calved (DOF group 163) on d 68 of the project and therefore was removed from the analysis as well. Following each groups allotted days on feed, heifers were humanely harvested at the Auburn University Lambert-Powell Meat Laboratory.

Carcass Evaluation

Following harvest, hot carcass weights (HCW) were taken for each heifer. Individual carcass evaluation was recorded on each carcass 24 h postmortem, which included USDA quality and yield grades. Carcasses were ribbed between the twelfth and thirteenth rib in order for trained carcass graders to record carcass traits for each animal (USDA, 1997). Carcass characteristics consisted of LM area; 12^{th}-rib fat thickness; % kidney; pelvic and heart (KPH) fat; and marbling scores.

Sample Preparation

Following carcass evaluation, boneless loins were removed from the right side of the carcass. Loins were vacuum-packaged and aged for 21 d at 4 ± 2^{o}C. Following the aging period, 7 steaks (2.54 cm) were cut from each loin starting from the anterior end. Three steaks used for a simulated retail display time (display) of 7 days for sensory analysis, Warner-Bratzler shear force, and analytical steak for TBARS analysis, respectively. Following cutting, these steaks were placed on a Styrofoam 4S size tray and overwrapped using polyvinyl chloride (PVC) film in order to go into retail display. The next 4 steaks were cut for fresh steaks for sensory panel, Warner-Bratzler shear force, analytical steak for TBARS analysis, and an extra steak, respectively. All fresh steaks were vacuum-packaged and stored in a -25^{o}C deep freezer until further analyses was completed.

Color Measurements

Steaks for display measurements were placed in a Tyler (Model DMG-8, Niles, MI) retail display chest case with a temperature of 4 ± 2°C. Color measurements were taken for 7 d using a Hunter Miniscan XE Plus (Hunter Laboratories Model MSXP-4500C, Reston, VA) using a 10° observation angle, D65 illuminant and 3.5-cm aperture. Color measurements were taken on the anterior side of the steak in two locations for an average measurement of L*, a*, and b*color values. Additionally, hue angle, saturation index and the 630/580 ratio (fresh meat color) ratio were calculated during retail display time. The 630/580 ratio was calculated according to Hunt (1988), which states that 630 nm is highest peak for oxymyoglobin and 580 nm is representative of lowest peak for oxymyoglobin on an isobestic curve.

Warner-Bratzler Shear Force

Warner-Bratzler shear force evaluation was conducted on both fresh and 7 d displayed steaks according to the procedures by the American Meat Science Association (AMSA, 1995). Prior to cooking, steaks were thawed for 24 h at 4 ± 2°C. After thawing, steaks were removed from vacuum packages, weighed, and placed on a preheated George Foreman clam-style grill (Model GRV120, Macon, MO). Using an Electro-Therm Digital theromometer (Model TM99A, Cooper Instruments Corp., Middlefield, CT), an internal temperature of 70°C was achieved before each steak was removed from the grill. Steaks were then placed on a Styrofoam tray, overwrapped with PVC film, and transferred back into a refrigerator (4 ± 2°C) in order to cool for 24 h. Following cooling, 6 cores (1.27 cm diameter) were then taken from each steak parallel to the muscle fibers. Warner-Bratzler shear force was measured on each core using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) and the mean shear force was calculated for each steak. The tests were performed using a Warner-Bratzler Probe and Guillotine set. The

probe was programmed to be lowered 30 mm after detection of resistance. A pre-test speed of 2.0 mm/s, penetration speed of 3.3 mm/s, and post-test speed of 10 mm/s was utilized during shearing. Each core was individually sheared perpendicular to the muscle fibers in the middle avoiding connective tissue in order to determine peak force (kg). An average of six cores were analyzed for each steak.

Trained Sensory Evaluation

Sensory evaluation was conducted as described by Cross et al. (1978) using a 7-member trained sensory panel. Steaks were thawed at 4 ± 2^{o}C for 24 h. After being removed from the vacuum package, steaks were cooked on a preheated George Foreman clam-style grill (Model GRV120, Macon, MO). After reaching an internal temperature of 70^{o}C, steaks were removed, subcutaneous fat was trimmed and cubes (1 cm^{3}) were cut and placed in a serving tray. Trays were kept in an incubator, preheated to 65^{o}C until all samples were prepared and panelists began evaluation.

Prior to beginning the evaluation, sensory panelists were trained to properly rate samples based on initial and sustained tenderness, initial and sustained juiciness, flavor intensity, and off-flavor descriptors. Panelists were placed in a sensory booth separated into individual cubicle and included a red incandescent light overhead. A cup of water and non-salted crackers were also included in individual cubicles for panelists to cleanse their palates. Panelists were asked to obtain two samples from each steak in order to rate samples on an 8-point hedonic scale (Appendix A). Panelists evaluated no more than 8 samples per day to avoid fatigue.

The sensory panel score sheet consisted of the following categories: initial and sustained juiciness, initial and sustained tenderness, flavor intensity, and off-flavor. The hedonic scale ranged from 1 to 8, with 1 being a negative trait and 8 being a positive trait for juiciness,

tenderness, and flavor intensity, where 1 = extremely dry, tough, bland and uncharacteristic of beef, and 8 = extremely juicy, tender, intense, and characteristic of beef, respectively.

Histochemical Fiber Typing

Fiber type staining was conducted using the techniques of Soloman and Dunn (1988) with modifications (Appendix B). Longissimus muscle samples from the twelfth rib were removed 24 h postmortem for fiber type analysis. Fiber type samples were then cut into 1.27 cm. x 0.64 cm. slices, frozen in isopentane, submerged in liquid nitrogen, vacuum packaged, and stored in a -80^{o}C ultra cold freezer until analysis. Samples were mounted on a cryostat chuck using liquid media freezing gel (TBS Tissue Freezing Medium #H-TFM; Durham, NC) and set for 15 min. Chucks were then mounted onto a Microm HM505E cryostat (Thermo Fisher Scientific, Waltman, MA) set at -20^{o}C, cut 10 µm thick, and placed onto a microscope slide (Fisherbrand Superfrost Excell Microscope Slides). Four meat sections were cut on the cryostat and placed on slides for each sample in order to type a representative number of fibers. After slides completed the staining procedure, they were removed from the slide holder and allowed to dry before mounting a cover slip using Glycerol mounting gel.

Typing of the fibers was performed using a Motic Type 102M microscope (Motic; Richmond, British Columbia). Six to eight pictures were taken from each sample in order to obtain a representative sample of 75-100 muscle fibers for each animal. The stained fibers were typed as either βR (red, oxidative), αR (intermediate, oxidative/glycolytic), or αW (white, glycolytic) by outlining the fibers on the picture. In addition, as the fiber was outlined, the area of the individual fiber was calculated and recorded.

Thiobarbituric Acid Reactive Substance (TBARS)

A modified method of Wang et al. (2002) was used to perform thiobarbituric acid reactive substance assay. Standards (0, 2, 4, 6, 8, 10, 20, 30 nM/mL) TEP/TCA were completed to calculate a regression line in order to calculate mg/mL malonaldehyde for all samples.

Thiobarbituric acid reactive substances were performed on a fresh (21 d aged) and a display (21 d age plus 7 d in retail display) steak. The procedure is shown in Appendix C. A representative 5 g sample of meat from each steak was cut into small pieces and placed in a 50 mL centrifuge tube and 15 mL of TCA was added to each tube and homogenized for 20-30 s using a Bella Cucina Rocket Blender (Virginia Highlands; Atlanta, GA). Following homogenation, samples were placed in a swinging bucket Beckman-Coulter Allegra X-15R centrifuge (Beckman Coulter, Inc.; Brea, CA) for 10 min at 1500 x *g*. Samples were then removed from the centrifuge and filtered using No. 4 Whatman paper into 16 mL test tubes. Samples were then loaded into 96 well microplates with a maximum capacity of 300 µL. The wells were filled with 125 µL of sample followed by 125 µL of TBA in triplicate. Standards were loaded in the first 3 columns. Two triplicate rows contained distilled water to serve as blanks. All samples were then incubated at 40^{o}C on a VWR microplate shaker for 130 min at a shaker speed of 100. Following incubation, samples were read on a Thermo Multiscan EX (Thermo Fisher Scientific, Waltman, MA) spectrophotometer at 540 nm and measured on a standard regression curve to determine mg/mL of malonaldehyde.

Statistical Design

Data were analyzed using the general linear models procedure in SAS (SAS Inst. Inc., Cary, NC, 2002). The experiment was set up as a completely randomized design. Fixed effects in this experiment were RAC (RAC or CON) and DOF (79, 100, 121, 142, 163). Analyses of

carcass traits, muscle fiber types, sensory evaluation, and WBSF were performed using the PROC GLM procedure in SAS. Least squares means were separated with Fishers protected LSD using the PDIFF option of SAS for significant main and interaction effects. Color data from the retail display period was analyzed as a repeated measure over the 7 d. A significant level of 5% was set for all analyses.

III. RESULTS

Carcass Traits

Least squares means for carcass characteristics are shown in Table 2. Initial weights were similar ($P > 0.05$) for treatment groups. Ractopamine supplementation did not ($P > 0.05$) have significant effects on carcass characteristics. Final weights were lighest ($P < 0.05$) in DOF group 79 and heaviest ($P < 0.05$) in DOF groups 121, 142, and 163. Final weight for DOF group 100 was the same as all DOF groups. Hot carcass weights were higher ($P < 0.05$) in 100, 121, 142 and 163 d groups compared to 79 d. Backfat thickness was lower ($P < 0.05$) in 79 and 100 d compared to 121 and 142 d. Backfat thickness in 163 d was similar ($P > 0.05$) to 79, 100, 121, and 142 d. There were no differences ($P > 0.05$) in LMA measurements among DOF. The percentage of KPH fat was higher ($P < 0.05$) after 121 and 142 DOF and lower ($P < 0.05$) after 79d. Yield grades were most favorable and statistically different in carcasses fed 79 and 100 d compared to all other DOF.

Marbling scores are shown in Figure 1. Marbling scores were higher ($P < 0.05$) in CON cattle for 79 and 100 d compared to RAC. As DOF increased from 121 to 142 d, RAC treated cattle had the higher ($P < 0.05$) marbling scores. There were no differences ($P > 0.05$) in marbling score for 163 d CON and RAC cattle.

Muscle Fiber Types

Least squares means for muscle fiber type analysis is shown in Table 3. The RAC main effect and RAC by DOF interaction did not have significant ($P > 0.05$; data not shown) effects on muscle fiber type area or percentage of fiber types within the LM. Intermediate fiber types

were larger ($P > 0.05$) in 121 d than 100 d treatment groups. Red and white fiber type areas were not affected ($P < 0.05$) by DOF. In addition, DOF had a significant effect of the distribution of fiber type percentages in the muscle. Red fiber type percentage was lowest ($P < 0.05$) in DOF groups 79 and 100 compared to 121 DOF. Additionally, red fiber type percentage was intermediate in value in samples taken from cattle fed 142 and 163 d. However, DOF groups 142 and 163 were not significantly different from any other DOF groups. As a result of the increase in red fiber type percentage, the percentage of white fibers decreased, and DOF group 121 had the lowest ($P < 0.05$) percentage of white fibers (28.0%) compared to 79 and 100 DOF groups. Days on feed did not affect ($P > 0.05$) the percentage of intermediate fiber types in the muscle.

Color

Control steaks for DOF groups 121, 142, and 163 had lower ($P < 0.05$; Table 4) L* values compared to 100 DOF. However, in RAC steaks, L* values were lower ($P < 0.05$) for DOF groups 121 compared to 142 and 163. The L* values for DOF group 142 CON and DOF group 100 RAC were similar to all other L* values. The lowest ($P < 0.05$) a* values are seen in DOF group 121 and 163 CON and DOF group 121 RAC. The highest ($P < 0.05$) a* values were seen in RAC 79 DOF. The b* values were lowest ($P < 0.05$) in DOF group 163 CON and highest ($P < 0.05$) for DOF group 79 CON and RAC. Similar b* values were seen among DOF groups 100 and 121 CON and DOF groups 100, 121, 142 and 163 RAC. Saturation index was lowest ($P < 0.05$) for DOF group 163 CON and highest ($P < 0.05$) in CON and RAC 121 DOF steaks. Hue angle was not affected ($P > 0.05$) by DOF or RAC.

The results for the ratio of fresh meat color are shown in Figure 2. The 630/580 ratio decreased ($P < 0.05$) as days in retail display increased. Values were highest ($P < 0.05$) on d 0 for DOF

group 79 in both RAC and CON treatments. On d 2, DOF group 79 RAC steaks were higher ($P < 0.05$) than all other treatment steaks. Also on d 2, DOF group 163 CON steaks had the lowest ($P < 0.05$) value. By d 6 of retail display, all 630/580 values were similar ($P > 0.05$) for steaks. Figure 4 represents the saturation index across DOF in a seven day retail display. On day 0, DOF group 79 had higher ($P < 0.05$) saturation index compared to 100, 121, 142, and 163 DOF treatments. As display time increased from d 1 to d 6, the saturation index decreased ($P < 0.05$) for all DOF groups. On d 2 and d 3 of retail display, DOF group 79 was higher ($P < 0.05$) than DOF group 163. From d 4 to d 6 in display, there were no differences ($P > 0.05$) in saturation index between DOF treatment groups.

There were no differences ($P > 0.05$; Figure 5) in hue angle between DOF treatment groups for d 0, d 1, and d 2 of retail display. On d 3 and d 4, DOF group 121 had higher ($P < 0.05$) hue angle measurements than DOF group 100. On display days 5 and 6, there were no differences ($P > 0.05$) in hue angle for all DOF treatment groups.

Results for a* values for DOF treatment groups in retail display are shown in Figure 6. On d 0 of display, a* values were highest ($P < 0.05$) in DOF group 79. There were no differences ($P > 0.05$) in a* values between DOF treatment groups from d 1 to d 6 of retail display. Redness of steaks decreased ($P < 0.05$) for all DOF treatment groups as days in retail display increased.

Once again, DOF group 79 had the highest ($P < 0.05$; Figure 7) b* values on d 0 of retail display. All b* values were similar ($P > 0.05$) for DOF groups on d 1 of display time. On d 2, b* values were higher ($P < 0.05$) in DOF group 79 and 100 compared to DOF groups 121, 142, and 163. Day 3 of retail display shows that b* values were highest ($P < 0.05$) in DOF groups 79 and 142. There were no differences ($P > 0.05$) in b* values on d 4 and d 6 of retail display. However, on d 5, DOF group 79 had higher ($P < 0.05$) b* values compared to DOF group 163.

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS results are shown in Figure 11. Malonaldehyde (mg/mL) content was the same for display and non-display in 79 and 121 DOF steaks. Display steaks had higher ($P < 0.05$) malonaldehyde content than non-displayed steaks for 100, 142, and 163 DOF groups.

Sensory Evaluation

Results from sensory evaluation are shown in Table 5. The main effect of RAC had no effect ($P > 0.05$; data not shown) on initial and sustained tenderness, initial and sustained juiciness, flavor intensity, and off-flavor. However, a RAC*DOF ($P < 0.05$) interaction shows that initial and sustained tenderness were highest ($P < 0.05$) in RAC 163 d compared to all other RAC and CON treatments. There were no interaction effects ($P > 0.05$) for initial juiciness, sustained juiciness, and flavor intensity.

Flavor intensity was lower ($P < 0.05$) in DOF groups 79, 100, 121, and 142 compared to DOF group 163 (Figure 8). Figure 9 shows the effect of display on the off-flavor ratings by the sensory panel. Steaks that were in a seven day retail display had higher ($P < 0.05$) off-flavor ratings compared to steaks that were not in display at any time.

Warner Bratzler Shear Force Evaluation

Results for Warner Bratzler shear force are depicted in Figure 10. Shear force measurements were not affected ($P > 0.05$; data not shown) by DOF or RAC. Shear force values were lowest ($P < 0.05$) for steaks put in retail display for seven days. Display steaks resulted in a mean shear force measurement of 3.32 kg whereas non-display steaks had a mean shear force of 3.89 kg.

IV. DISCUSSION

Carcass Traits

The effect of RAC on carcass traits has been well documented in both steers and heifers. Being that RAC is a repartitioning agent, it would be expected that fat thickness would decrease in the carcass and LM area potentially would be increase. Walker et al. (2006) showed no differences in LM area, 12th-rib fat thickness, USDA yield grade, and marbling scores. However, Walker et al. (2006) did see an increase in HCW and KPH fat % with the addition of RAC into the diet. Results from a similar study by Schroeder et al. (2004a), showed no response to RAC for 12-rib fat thickness, LM area, marbling, yield grade and quality grade in feedlot heifers. Similar to the study by Walker et al. (2006), Schroeder et al. (2004a) showed an increase in HCW for heifers fed RAC.

Results for the current study also show no differences in LM area, 12th-rib fat thickness, USDA yield grade, and marbling score as affected by RAC. However, there were also no differences in HCW, which differs from studies shown above. Similar to the current study, Quinn et al. (2008) reported results for heifers fed a 200 mg·hd^{-1}d^{-1} supplement of RAC and showed no differences in carcass characteristics for treated animals. Several more reports showed that there were no significant differences in carcass traits for animals treated with RAC (Winterholler et al., 2006; Gruber et al., 2007; Strydom et al. 2008).

The lack of differences in carcass characteristics as affected by RAC supplementation from the current study is most likely attributed to the similarity in final weights. In addition, the

high initial weights most likely had an effect on quality results as well. The initial weights of the animals were near 450 kg. On average, medium to large frame heifers expected to grade choice typically reach slaughter weight between 450 and 520 kg (1000-1150 lb.; USDA, 2000). Therefore, the animals receiving RAC the final 35 days of feeding in this study were quite large by harvest time and exceeded the average market weight.

Muscle Fiber Types

Fiber type results in this experiment differ from other studies that reported both fiber type area and fiber type percentages are affected by RAC supplementation. Strydom et al., (2009) showed no difference in area of red fibers, but increases in both intermediate and white fiber types for steers treated with 300 mg·hd·$^{-1}$d^{-1} RAC. Gonzalez et al. (2007) reported results in cull cows fed RAC and showed an increase Type I fiber area, but no increase in Type II fiber area. The current study agrees with Gonzalez et al. (2008), which showed no differences in area of longissimus muscle fiber types for cull cows. Ractopamine supplementation at a rate of 300 mg·hd·$^{-1}$d^{-1} did not result in a different response than a dosage of 200 mg·hd·$^{-1}$d^{-1} (Gonzalez et al., 2008).

The current study also agrees with studies (Gonzalez et al., 2006; Strydom et al., 2009) which examined the effect of RAC supplementation on fiber type distribution. Results from these studies showed that RAC supplementation had no effect on fiber type distribution. These studies differ from a study performed by Gonzalez et al. (2008), which showed a decrease in Type I and increase in Type IIA fiber percentage with RAC supplementation at 100, 200, and 300 mg·hd·$^{-1}$d^{-1}. However, Gonzalez et al., (2008) supplemented RAC in cull cows, which most likely is the reason for a differential response compared to animals in the current study. It is known that the growth plane of a young heifer and a cull cow have large differences and will most likely result in fiber distribution differences as well.

Color

Color measurements are often taken during retail display in order to show the deterioration of redness as storage time increases. Typically, as redness decreases during display time, an increase in metmyoglobin is seen because of oxidation of oxymyoglobin (Aberle et al., 2001). It has been noted that color is the single most important attribute that consumers assess when preparing to make a meat purchase (Hedrick et al., 1994).

Although there is great importance associated with meat color, there have not been many studies reporting the effects of RAC supplementation on the shelf-life of steaks. Avendanos-Reyes (2006) showed that over a 14 d display time, there were no differences for L*, a*, and b* for RAC treated and control steaks. Therefore, these authors concluded that RAC does not affect color measurements. Another experiment performed by Quinn et al. (2008) showed no significant differences in L*, a*, and b*over a 7 d retail display time in heifers fed 200 mg/hd/d RAC the final 28 d of feeding. Similarly, Gonzalez et al. (2009) reported no differences in L*, a*, and b* for steers supplemented with 200 $mg \cdot hd^{\cdot -1}d^{-1}$ RAC. The current study also showed that the main effect of RAC and DOF did not contribute to differences in color measurements of retail displayed steaks. As expected, there was color deterioration as days in display increased.

TBARS

Similar to color deterioration, the oxidation of lipids in meat during retail display is typically increased. Therefore, TBARS are often assessed to measure the amount of malonaldehyde that is produced as lipid deterioration increases. In this experiment, the main effect of RAC had no effect ($P > 0.05$) on TBARS results. A study by Apple et al., (2008) showed similar results in pigs, where RAC did not have an effect on malonaldehyde production. An interesting result from this data, shows that malonaldehyde production was similar for DOF groups 79, 100 and 121 in

both display and non-displayed steaks. However, DOF groups 142 and 163 display steaks had a higher amount of malonaldehyde production, suggesting that increased DOF decreases lipid stability in steaks.

Warner- Bratzler Shear Force

Several studies have shown contrasting results to the effect of RAC on shear force measurements. Gruber et al. (2008) showed higher WBSF for steers treated with a 200 mg·hd·$^{-1}$d^{-1} supplementation of RAC compared to control steers. Similarly, Avendano-Reyes et al. (2006) showed an increase in shear force measurements from 4.40 to 4.83 kg for steers supplemented with 300 mg·hd·$^{-1}$d^{-1} RAC. Schroeder et al. (2004) reported no differences in WBSF for cattle treated with 100 and 200 mg·hd·$^{-1}$d^{-1} RAC, but higher WBSF at 300 mg·hd·$^{-1}$d^{-1} dosage.

In the current study, shear force measurements were not influenced by RAC. This is concurrent with Quinn et al. (2008), who showed no differences in shear force for control and treated (200 mg·hd·$^{-1}$d^{-1}) heifers. Typically, RAC is shown to be a less potent β-agonist, thus explaining the reason for insignificant changes in meat tenderness. Strydom et al. (2009) also showed no differences in shear force for control and RAC treatment in feedlot steers.

In the current study, shear force was lower for steaks that were in 7 d retail display. These results can potentially be attributed to the increased aging time postmortem that the steaks in display had. Steaks that were frozen immediately after cutting had a 21 d postmortem aging time, whereas those in display had an additional 7 d aging time. These results agree with other studies, such as Strydom et al. (2009) who showed continuous decrease in shear force measurements after 2, 7, and 14 d.

Sensory Evaluation

Gruber et al. (2008) examined the effects of 200 mg·hd·$^{-1}$d^{-1} RAC on cattle of differing biological type. Sensory evaluation from this experiment showed differences in tenderness ratings from the cattle treated with RAC. Similarly, Schroeder et al. (2004b) reported that there were no differences in tenderness ratings for cattle treated with a dosage below 300 mg·hd·$^{-1}$d^{-1}. However, at the dosage level of 300 mg·hd·$^{-1}$d^{-1}, tenderness ratings were different between the treated and control groups. These authors interpreted the differences between 300 mg·hd·$^{-1}$d^{-1} treated and control steaks to be non-detectable by consumers. Therefore, this study concluded that RAC has no effect on palatability characteristics. In the current study, there were no differences in sensory attributes as affected by RAC supplementation.

Initially, it was shown that β-agonists had a negative effect on tenderness attributes, which was shown through shear force and sensory evaluation. However, recent studies comparing effects of individual β-agonists have shown that RAC may not exhibit the same negative results on tenderness as other β-agonists. Koohmaraie et al. (1991) and Geesink et al. (1993) reported that a decrease in tenderness in β-agonists such as clenbuterol, cimaterol, and L-644,969 can be attributed their classification as a β_2-agonist. β_2-agonists have been shown to have increased levels of calpastatin, which results in a decrease in postmortem degradation (Koohmaraie et al., 1991). Therefore, a decrease in tenderness measurements is expected. Ractopamine differs from the β_2-agonists, because it is classified as a β_1-agonist. The current study suggests that β_1-agonists such as RAC, do not impart negative effects on postmortem degradation and meat quality, based on the receptor subtype.

Implications

The current study shows that RAC supplementation in yearling heifers does not have adverse effects on meat quality. The lack of differences between treatments in carcass characteristics is

likely due to the large initial weights of the animals. These results show that the addition of 300 $mg \cdot hd \cdot^{-1} d^{-1}$ of RAC the final 35 d prior to slaughter into the diet of yearling heifers does not positively or negatively affect meat quality attributes.

V. TABLES AND FIGURES

Table 1: Diet Composition fed to yearling heifers[1]

Ingredient	Percentage
Big Screen Corn	38.5
Wheat Midds	6.5
Corn Gluten Pellets	17.5
Dried Distillers Grain	9.5
Cottonseed hull pellets	10.0
Cottonseed hulls	5.0
Limestone	1.25
Soyhulls	6.5
Salt	0.5
Vitamins A,D,E	0.1
BICARB	1.0
Trace Minerals	0.1
Rumensin 80	0.019
Molasses	2.5
Fat	1.0

[1]As calculated: DM = 90.15%; CP = 13.66%, NDF = 32.17%, ADF = 15.72, NEm = 1.48 Mcal/kg, NEg = 0.76 Mcal/kg

Table 2: Least squares means ± SEM of carcass characteristics for heifers as influenced by RAC across five days on feed

	RAC			Days on Feed[x]					
	CON	RAC	P[4]	79	100	121	142	163	P
No. An.[1]	35	36	-	16	16	16	16	7	
In. Wt.[2], Kg	430.9 ± 14.1	433.0 ± 14.9	NS	436.3 ± 20.4	435.6 ± 20.4	434.3 ± 20.4	433.4 ± 20.4	421.0 ± 31.1	NS
Final Wt.[3], Kg	570.4 ± 19.4	577.1 ± 20.5	NS	532.4^{a} ± 28.1	566.1^{ab} ± 28.1	584.6^{b} ± 28.1	588.7^{b} ± 28.1	596.9^{b} ± 42.9	0.0114
HCW, kg	347.5 ± 13	353 ± 13	NS	321.4^{a} ± 18	345.6^{b} ± 18	362.1^{b} ± 18	364.8^{b} ± 18	357.9^{b} ± 18	0.003
BF, in	1.75 ± 0.05	1.78 ± 0.05	NS	1.34^{a} ± 0.07	1.32^{a} ± 0.07	2.00^{b} ± 0.07	2.31^{b} ± 0.07	1.85^{ab} ± 0.07	0.0003
LM area, cm^2	84.4 ± 1.94	85.8 ± 1.94	NS	81.9 ± 1.94	85.2 ± 1.94	86.5 ± 1.94	89.6 ± 1.94	82.6 ± 3.61	NS
KPH, %	3.29 ± 0.01	3.32 ± 19.4	NS	2.25^{a} ± 0.2	3.60^{bc} ± 0.2	3.95^{c} ± 0.2	3.66^{c} ± 0.2	3.04^{b} ± 0.3	<0.0001
YG	3.62 ± 0.2	0.01 ± 0.2	NS	2.91^{a} ± 0.2	3.17^{ab} ± 0.2	4.0^{c} ± 0.2	4.10^{c} ± 0.3	3.86^{bc} ± 0.4	0.0024

[x] Mean ± S.E.
[abcd] Means within a row lacking a common superscript differ significantly ($p < 0.05$)
[1] Number of animals
[2] Initial Weight
[3] Final Weight
[4] P-Value

Table 3: Least squares means ± SEM of mean fiber type areas (µm) and fiber type percentages for heifers across days on feed

		DOF					
		79	100	121	142	163	P-value
Area, (µm)							
	Red	2365.5 ± 113	2352.7 ± 109	2674.6 ± 113	2715.9 ± 132	2474.2 ± 219	NS
	Inter	3341.3^{ab} ± 154	2917.8^{a} ± 148	3672.5^{b} ± 153	3304.3^{ab} ± 179	3111.5^{ab} ± 297	0.02
	White	4243.4 ± 192	4085.2 ± 186	4708.8 ± 192	4163.8 ± 225	3965.6 ± 371	NS
Type, %							
	Red	34.3^{a} ± 1.8	34.1^{a} ± 1.8	43.7^{b} ± 1.8	40.9^{ab} ± 2.1	41.3^{ab} ± 3.4	0.0008
	Inter	30.5 ± 1.6	31.1 ± 1.5	28.2 ± 1.6	27.4 ± 1.9	26.6 ± 3.1	NS
	White	35.2^{b} ± 1.7	34.8^{b} ± 1.7	28.0^{a} ± 1.7	31.7^{ab} ± 2.0	32.1^{ab} ± 3.3	0.03

[ab] Means lacking a common superscript within a row differ significantly ($p < 0.05$)

Table 4: Least squares means ± SEM of color measurements as affected by RAC across five days on feed

		L*	a*	b*	Hue Angle	Saturation[1]
CON						
	79	$40.1^{bc} \pm 0.9$	$16.7^{bc} \pm 0.8$	$18.0^{d} \pm 0.4$	49.5 ± 1.3	$24.9^{ef} \pm 0.7$
	100	$41.1^{c} \pm 0.8$	$15.9^{bc} \pm 0.7$	$16.0^{bc} \pm 0.4$	47.4 ± 1.1	$22.9^{bcde} \pm 0.6$
	121	$36.5^{a} \pm 0.8$	$13.5^{a} \pm 0.7$	$15.9^{bc} \pm 0.4$	52.5 ± 1.1	$21.2^{b} \pm 0.6$
	142	$39.4^{abc} \pm 1.4$	$16.5^{bc} \pm 1.1$	$17.2^{cd} \pm 0.6$	48.4 ± 2.0	$24.1^{def} \pm 1.1$
	163	$37.5^{ab} \pm 1.3$	$12.6^{a} \pm 1.1$	$13.4^{a} \pm 0.6$	49.7 ± 1.9	$18.7^{a} \pm 1.0$
RAC						
	79	$37.6^{ab} \pm 1.4$	$18.2^{c} \pm 1.2$	$18.4^{d} \pm 0.7$	47.4 ± 2.0	$26.3^{f} \pm 1.1$
	100	$38.1^{abc} \pm 1.4$	$16.0^{bc} \pm 1.2$	$15.4^{b} \pm 0.6$	46.5 ± 2.0	$22.5^{bcde} \pm 1.1$
	121	$36.3^{a} \pm 0.8$	$13.7^{a} \pm 0.7$	$16.1^{bc} \pm 0.4$	52.3 ± 1.1	$21.4^{bc} \pm 0.6$
	142	$39.5^{bc} \pm 0.8$	$15.8^{bc} \pm 0.7$	$16.6^{bc} \pm 0.4$	48.2 ± 1.1	$23.1^{cde} \pm 0.6$
	163	$39.7^{bc} \pm 1.1$	$14.6^{ab} \pm 0.9$	$15.8^{bc} \pm 0.5$	49.5 ± 1.6	$21.8^{bcd} \pm 0.9$
P-value		0.0269	<0.0001	<0.0001	0.2117	0.0008

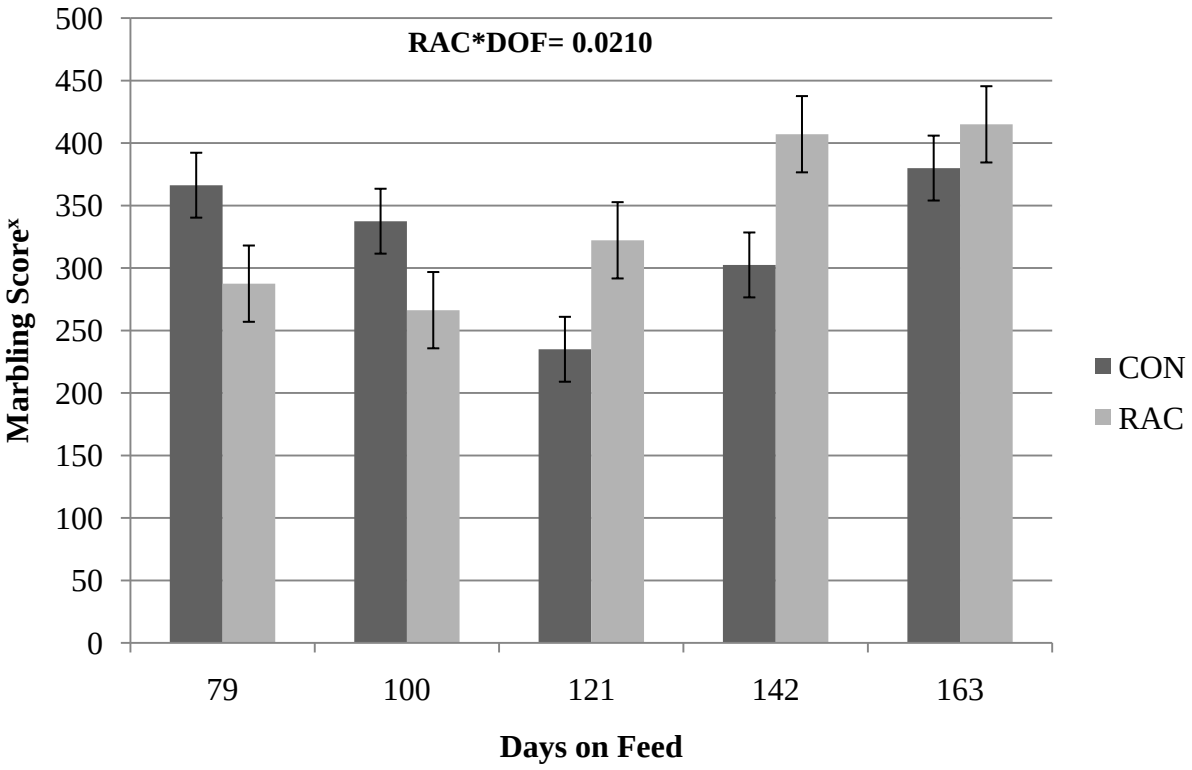
[abcd] Means lacking a common superscript within a column differ significantly ($p < 0.05$)
[1] Saturation Index

Table 5: Least squares means ± SEM of sensory characteristics for RAC and CON steaks across days on feed

		IT[1]	ST[2]	IJ[3]	SJ[4]	FI[5]
CON						
	79	5.44[a] ± 0.15	5.31[a] ± 0.16	5.31 ± 0.17	5.05 ± 0.18	4.85 ± 0.12
	100	5.42[a] ± 0.13	5.25[a] ± 0.14	5.26 ± 0.15	5.16 ± 0.15	4.87 ± 0.11
	121	5.14[a] ± 0.14	5.07[a] ± 0.14	5.01 ± 0.15	4.90 ± 0.15	4.91 ± 0.11
	142	5.35[a] ± 0.13	5.26[a] ± 0.14	5.35 ± 0.15	5.15 ± 0.16	4.64 ± 0.11
	163	5.39[a] ± 0.24	5.15[a] ± 0.26	5.46 ± 0.28	5.36 ± 0.28	5.26 ± 0.20
RAC						
	79	5.07[a] ± 0.13	4.93[a] ± 0.14	5.15 ± 0.16	4.93 ± 0.16	4.61 ± 0.11
	100	5.06[a] ± 0.13	4.90[a] ± 0.14	5.02 ± 0.15	4.85 ± 0.15	4.70 ± 0.11
	121	5.27[a] ± 0.12	5.23[a] ± 0.13	5.31 ± 0.14	5.23 ± 0.15	4.86 ± 0.10
	142	5.08[a] ± 0.14	5.15[a] ± 0.15	5.22 ± 0.16	5.20 ± 0.17	4.79 ± 0.12
	163	6.19[b] ± 0.18	5.94[b] ± 0.20	6.12 ± 0.22	5.83 ± 0.22	5.10 ± 0.16
P-value		0.0058	0.0159	0.1166	0.1670	0.4911

[ab] Means lacking a common superscript within a column differ significantly ($p < 0.05$)
[1] Initial Tenderness
[2] Sustained Tenderness
[3] Initial Juiciness
[4] Sustained Juiciness
[5] Flavor Intensity

Figure 1: Least Squares Means of marbling scores in yearling heifers as affected by RAC across five days on feed



[x] Marbling scores were determined according to the USDA grading standards using a scale of: traces= 0-99; slight = 100-199; small = 200-299; modest = 300-399; moderate = 400-499; slightly abundant = 500-599; moderately abundant = 600-699; abundant = 700-79

Figure 2: Least squares means of 630/580 ratio of steaks displayed for 7 d as influenced by RAC, days on feed, and display day

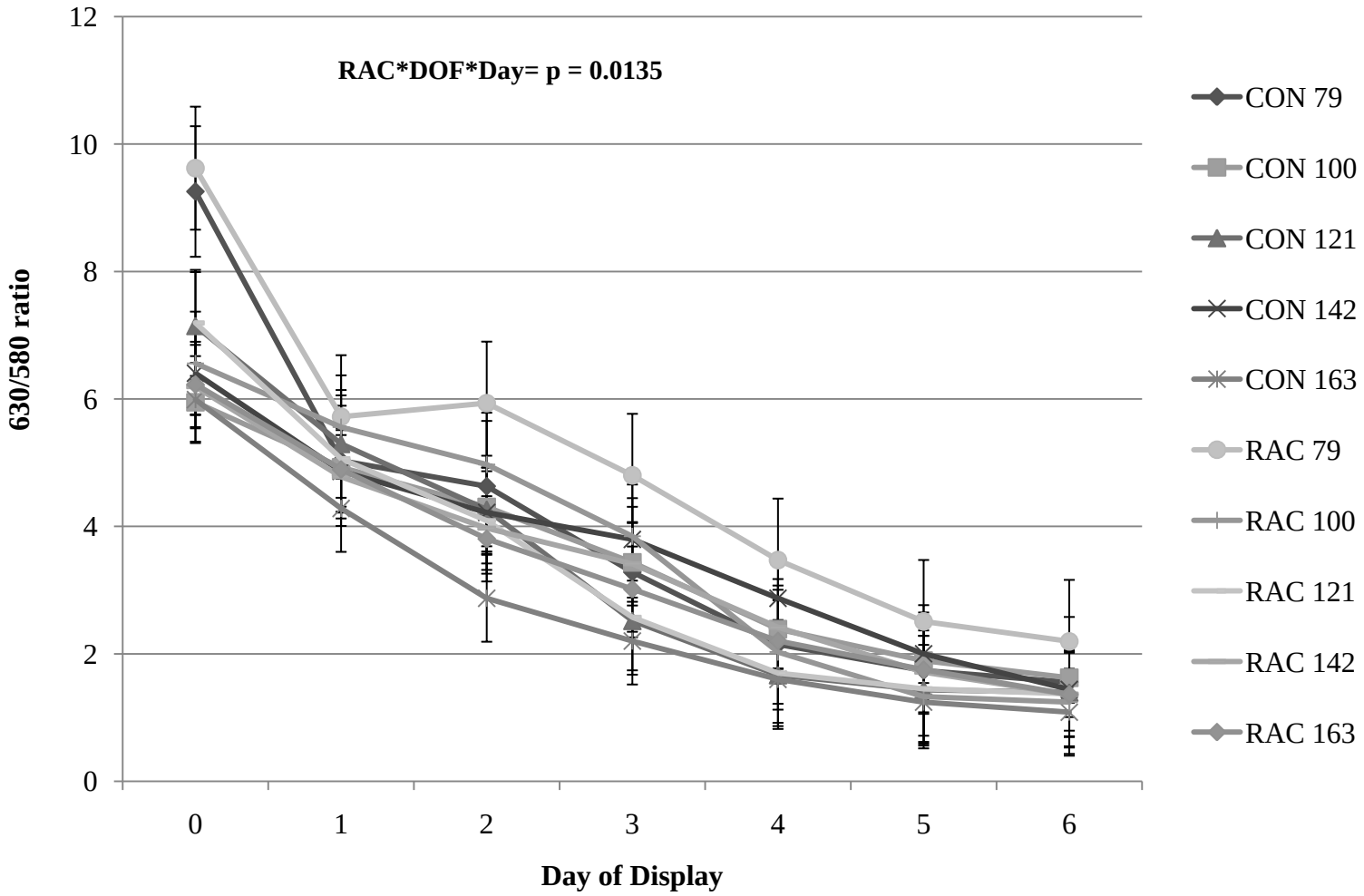


Figure 3: Least squares means of saturation index across DOF in a seven day retail display time

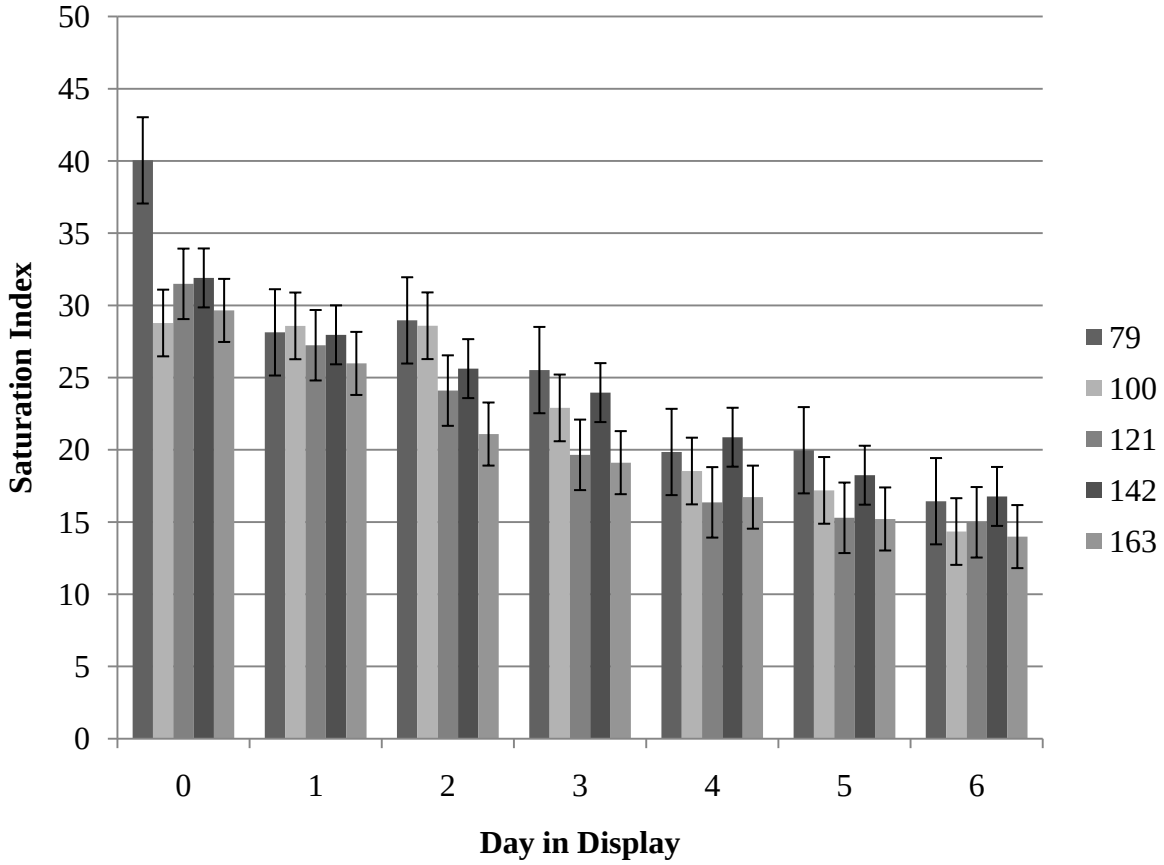


Figure 4: Least squares means of hue angle across DOF in a seven day retail display time

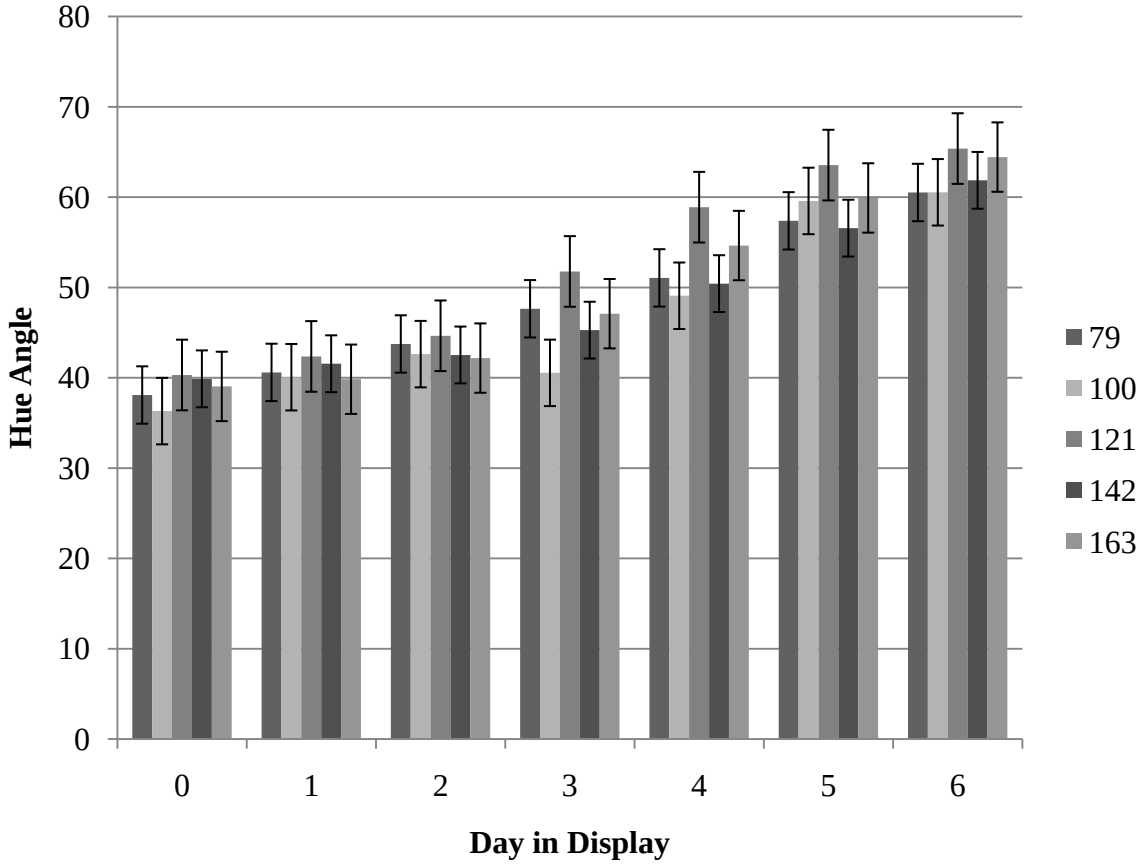


Figure 5: Least squares means of a* values across DOF in a seven day retail display time

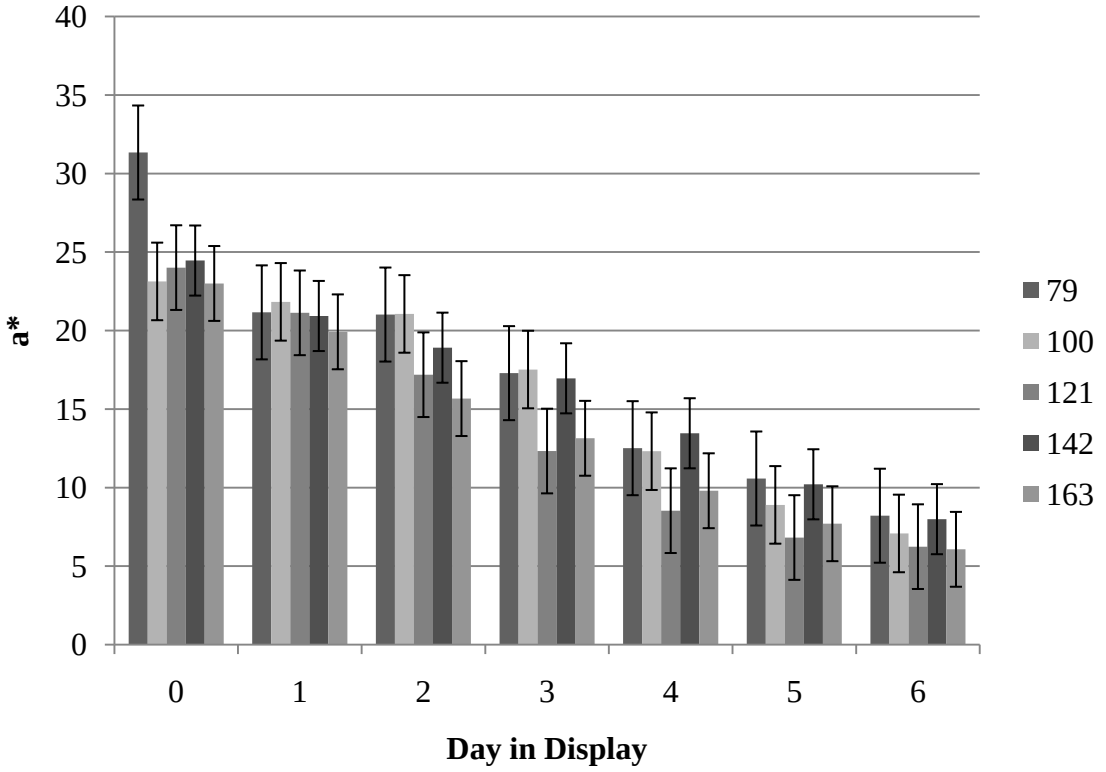


Figure 6: Least squares means of b* values across DOF in a seven day retail display time

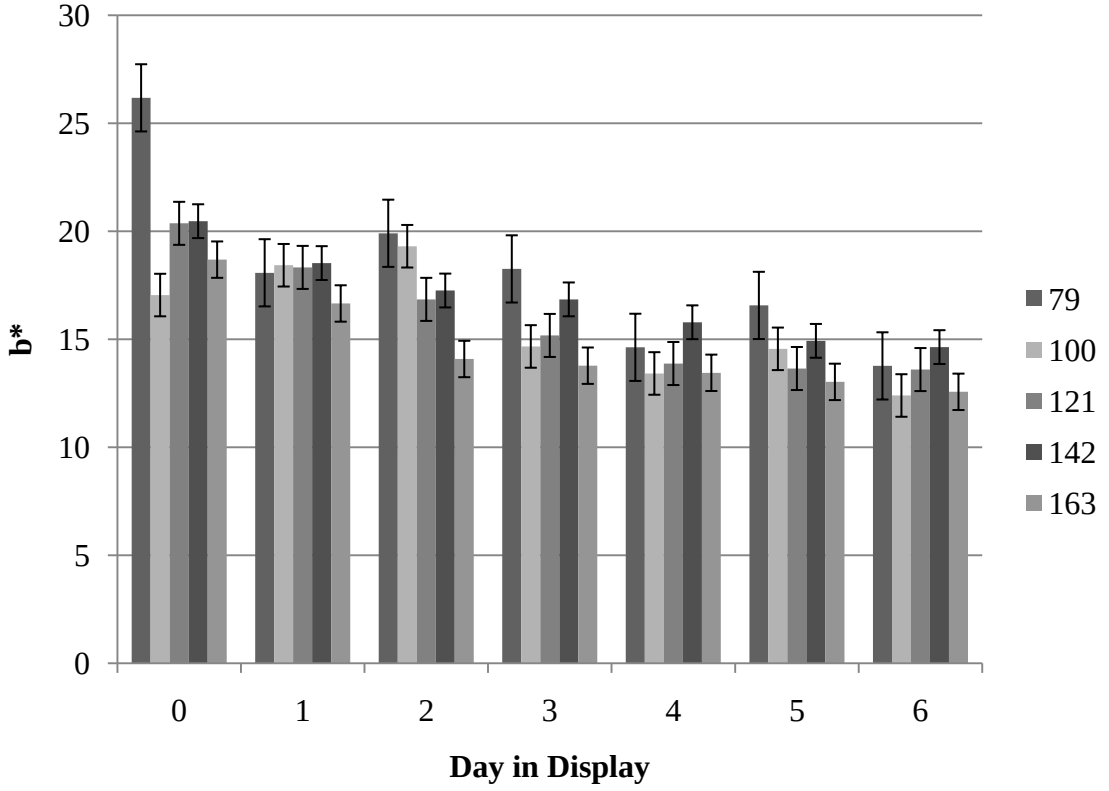


Figure 7: Least squares means of Thiobarbituric Acid Reactive Substances (TBARS) of steaks displayed and not displayed

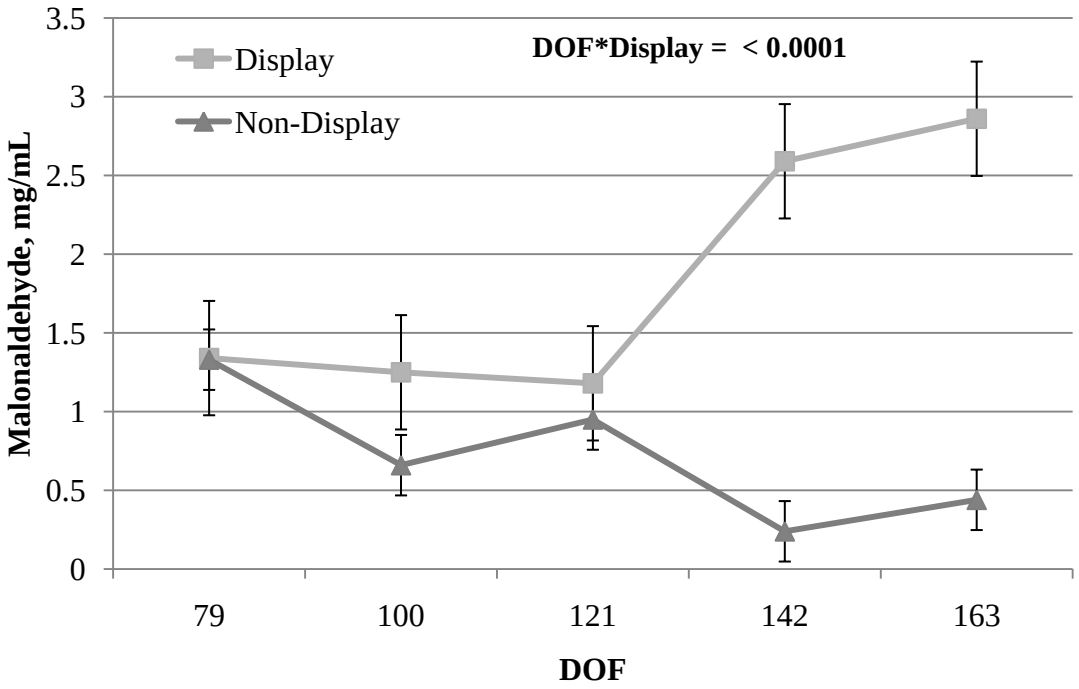


Figure 8: Least squares means of flavor intensity for steaks across five days on feed

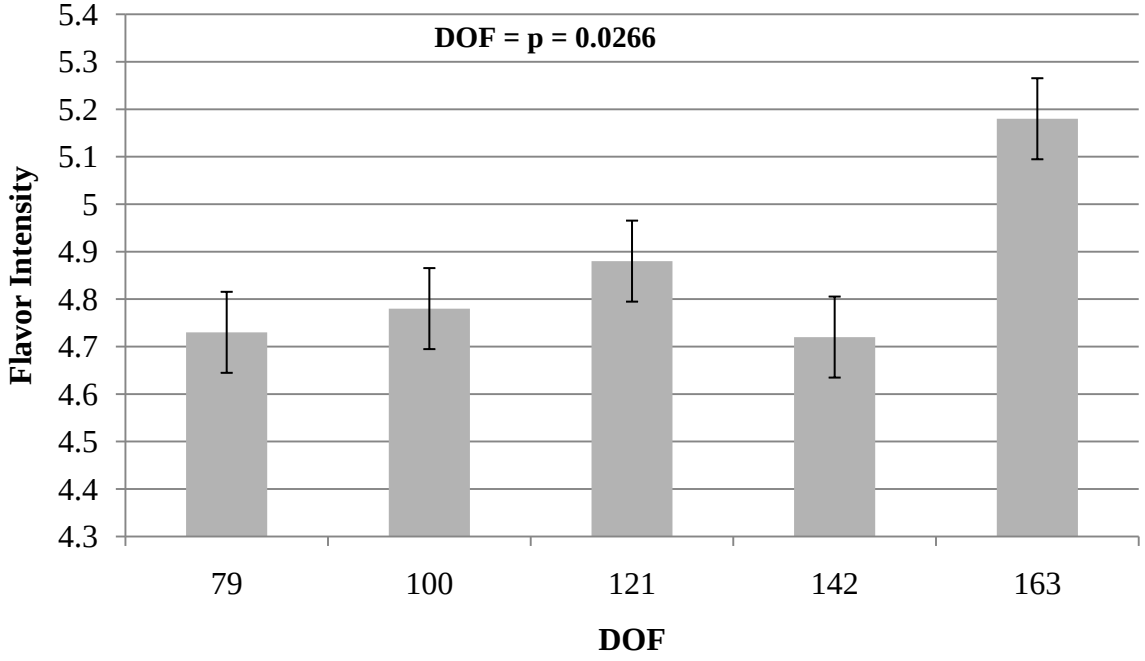


Figure 9: Least squares means of off-flavor of steaks not in display and steaks after a 7 day retail display time

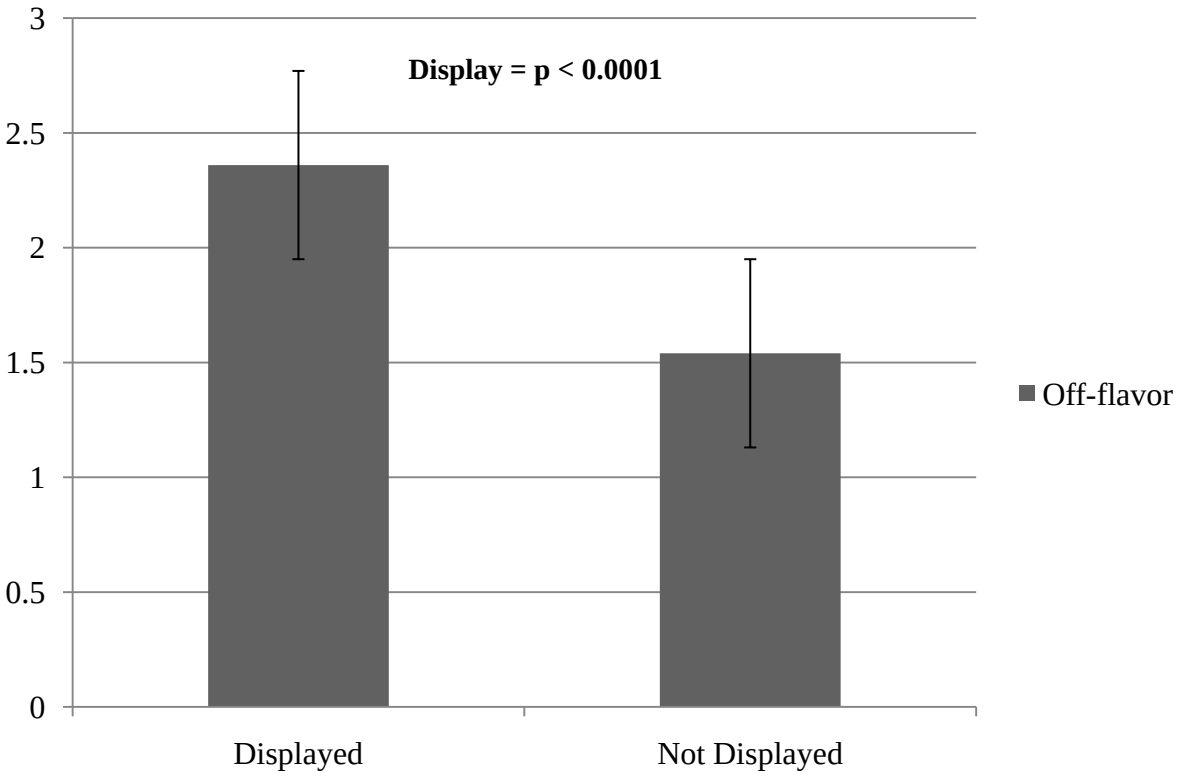
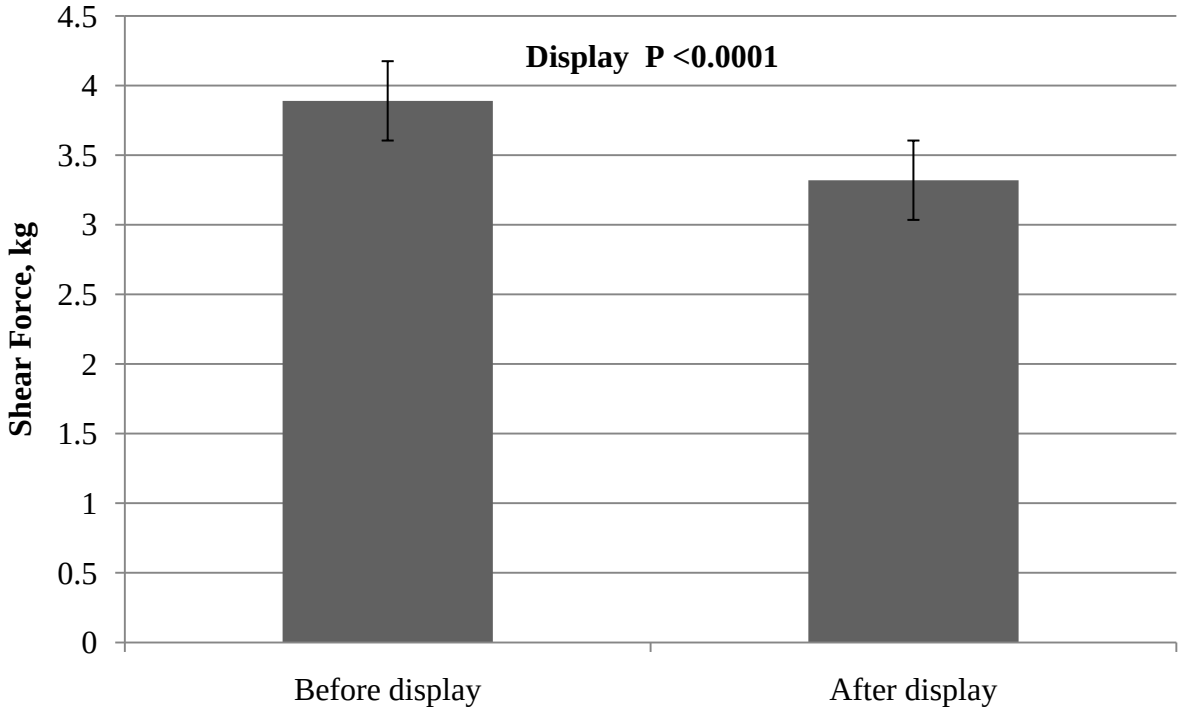


Figure 10: Least squares means of Warner Bratzler Shear Force for steaks influenced by 7 day retail display time



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APPENDICES

APPENDIX A.

SENSORY PANEL SAMPLE SHEET

Sample	Initial Juiciness	Sustained Juiciness	Initial Tenderness	Sustained Tenderness	Flavor Intensity	Off-Flavor	OFF Descriptor

Juiciness	Tenderness	Flavor Intensity	Off-Flavor	Off-flavor descriptors
8=Extremely Juicy	8=Extremely Tender	8=Extremely Intense	8=Extreme Off	8= Metallic
7=Very Juicy	7=Very Tender	7=Very Intense	7= Intense off	7=Salty
6=Moderately Juicy	6=Moderately Tender	6=Moderately Intense	6=Very off	6=Livery
5=Slightly Juicy	5=Slightly Tender	5=Slightly Intense	5=Moderate Off	5=Grassy
4=Slightly Dry	4=Slightly Tough	4=Slightly Bland	4=Modest Off	4=Bitter
3=Moderately Dry	3=Moderately Tough	3=Moderately Bland	3=Small Off	3=Bloody
2=Very Dry	2=Very Tough	2=Very Bland	2=Slight Off	2=Rancid
1=Extremely Dry	1=Extremely Tough	1=Extremely Bland	1=No off flavor	1=Other-Explain

APPENDIX B.

Fiber Typing Procedure
Combined Stain For Identifying Muscle Fiber Types

Principle:

Unfixed tissue sections are stained first for metabolic function NADH and then for physiological function by the acid ATPase reaction.

Reference:

Simultaneous histochemical determination of 3 fiber types in single sections of ovine, bovine, and porcine skeletal muscle. M.B. Solomon and M. C. Dunn. J. Anim. Sci. 1988. 66:255-264.

Comparative aspects of muscle fiber types in different species. C.R. Ashmore and L. Doerr. 1971. Exp. Neurol. 31:408.

Solutions:

1. Acid Pre-incubation Solution:
   - 100 ml ***$CaCl_2$ (0.18 M)***
   - 3 ml ***Glacial acetic acid***
   - 890 ml ***ddH_2O***
   - Adjust pH to 4.15, QS 1 L

   Note: Even though the pH is adjusted at this step it will need to be adjusted again depending on the species being typed.
2. Rinse
   - 12.1g ***Tris Base***
   - 100 ml ***$CaCl_2$ (0.18 M)***
   - 900 ml ***ddH_2O***
   - Adjust pH to 7.8
3. ATPase Incubation Medium: (Make fresh immediately before use)

	40 ml batch	200 ml batch
2-Amino 2-Methyl 1-Propanol (1.5 M)	2.68 ml	13.40 ml
$CaCl_2$	4.0 ml	20.00 ml
KCl	0.148 g	0.740 g
ATP	0.0608 g	0.304 g
ddH_2O	32.0 ml	160.0 ml
	(QS to 40 ml)	(QS to 200 ml)

   Adjust pH to 9.4; incubate tissues to 37^{o}C

4. *B-NADH* Incubation Solution ** (Make fresh daily)**
   10 ml ***0.2 M Tris buffer***, pH 7.4
   10 mg ***Tetranitro Blue Tetrazolium (TNBT)***
   8 mg ***Nicotineamide Adenine Dinucleotide, reduced***

   NOTE: No matter how long you mix this it never seems to go into solution. Therefore, it *MUST be filtered*

5. Tris Buffer, pH 7.4:
   75 ml ***0.2 M Tris base (12.11 g/500 ml)***
   126 ml ***0.1 M HCl (50 mls of 1 N HCl, QS 500 ml)***
   ** (***1N HCL*** is 41.7ml HCL/458ml ddH_20)**
   174 ml ***ddH_2O***
   As a preservative, add a few drops of ***Chloroform***.

6. Ehrlich's Hematoxylin Stain:
   6 g ***Hematoxylin***
   300 ml ***Ethyl alcohol, absolute***
   9 g ***Aluminum Ammonium Sulfate***
   300 ml ***ddH_2O***
   300 ml ***Glycerin (Glycerol)***
   0.72 g ***Sodium Iodate***
   30 ml ***Glacial Acetic Acid***
   Mix & filter (**NOTE:** The stain can be reused several times)

7. ***0.18 M $CaCl_2$*** : 26.46 g/L

8. ***1% (w/v) $CaCl_2$*** : 10 g/L

9. ***2% (w/v) $CoCl_2$*** : 10 g/500 ml

10. ***2% (v/v) Ammonium Sulfide***: **(Must mix under the hood)**
10 ml/500 ml

11. ***50% (v/v) Ethyl Alcohol***:
250 ml in 250 ml ***distilled H_2O***

12. ***Glycerol Gelatin*** /**Fluoromount-G** (to mount slides):
The glycerol needs to be warmed before use. Keep a beaker of water on a hot plate (lowest setting) and keep the glycerol bottle in the beaker while mounting slides.

Staining Sequence for Bovine:

1. β-NADH solution, 45 min. @ 37^oC.

2. Distilled water, 30 sec.
3. Acid pre-incubation solution, **pH 4.30** ; 10 min.
4. Rinse solution, pH 7.8; 1 min.
5. Rinse solution, pH 7.8; 1 min.
6. ATPase incubation solution, pH 9.4; 30 min. @ 37^{o}C.
7. 1% $CaCl_2$, 30 sec.
8. 1% $CaCl_2$, 30 sec.
9. 1% $CaCl_2$, 30 sec.
10. 2% **Co**Cl_2, 3 min.
11. Distilled water, 30 sec.
12. Distilled water, 30 sec.
13. Distilled water, 30 sec.
14. Distilled water, 30 sec.
15. 2% Ammonium Sulfide, 3 min. **(Do this step in the hood)**
16. Running distilled water, 3 min.
17. Hematoxylin, 5 min.
18. Running distilled water, 3 min.
19. 50% EtOH, 2 min.
20. Drain and mount with Glycerol gel.

Results:

β-Red Fibers: Fibers that stain dark purple

α-Red Fibers: Fibers that stain intermediate

α-White Fibers: Fibers that stain light purple

Note:

- For "cleaner" slides, filter the NADH solution before using it.
- If 3 fiber types are not identifiable, increase the pH of the acid pre-incubation solution until desired intensity is obtained.

Appendix C.

Thiobarbituric Acid Reactive Substances Assay

Extraction solution (TCA)
7.5% TCA (7.5 g/100 ml ddH2O or 75 g/L))
0.1% EDTA (0.1 g/100 ml ddH20 or 1 g/L)
0.1% Propyl Gallate (0.1 g/100ml ddH20 or 1 g/L)

Standard (TEP)
80 nM/ml Tetraethoxypropane (TEP)

TBA
1.15 g TBA in 100 mL

Standards:
235 µL of TEP into 1000 mL water= 1 µM/ml (80 nm/mL)
2 ml of TEP into 23 ml water= 40 nm/ml

	TEP (µL)	TCA (µL)	Pipette Setting
0	0	2000	1000 x 2
2	50	1950	975 x 2
4	100	1900	950 x 2
6	150	1850	925 x 2
8	200	1800	900 x 2
10	250	1750	875 x 2
20	500	1500	750 x 2
30	750	1250	625 x 2

Make standards in 16 ml tubes , VORTEX
Pipette 125 µL in first 3 columns of well.

Procedure:

- Mince and weigh 5 g of sample into a 50 mL centrifuge tube and add 15 mL TCA
- Homogenize for 20-30 s
- Centrifuge for 10 min. @ 1500 x g
- Filter with Whatman #4 into 16 ml glass tubes
- Load into 96 well plates by adding 125 µl sample and 125 µl TBA to each well. (Load each sample and standard in triplicate) VORTEX samples and standards before loading.
- Incubate microplate in shaker for 130 min at a shaker speed of 100, 40 C
- Read on spec at 540 nm
- Average three samples and create regression line.
- Average of the three samples is put into regression equation to determine mg/ml