EFFECT OF RACTOPAMINE-HYDROCHLORIDE ON MUSCLE FIBER MORPHOMETRICS, SATELLITE CELL POPULATION, AND SHELF-LIFE PROPERTIES OF BEEF CATTLE

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2008

To my love, Sara, and my Texas and Florida families. Without each of you I could not have finished this.

ACKNOWLEDGMENTS

I am glad that dissertations are not required reading for most people, because I know that I will forget to thank some people. If I forget to mention you, please know that I am not ungrateful, but that there have been a ton of people in my life that have helped me get to this point and it would be impossible to remember you all. To begin, I would like to first thank my supervisory committee. To the Chair of my committee, Dr. D. Dwain Johnson, I would like to thank you for giving me the opportunity to come to the University of Florida and work under you. I really appreciate the opportunity because I know that I was not the strongest meat scientist coming out of my Master's, but you allowed me the chance to further my education here. You have taught me valuable lessons in how to conduct research, teach and interact with students, and follow through on my research ideas. I consider it an honor to have worked under you. Appreciation is also extended to Dr. Sally Williams and Dr. J.P. Emond. Thank you for taking the time to serve on my supervisory committee and for bringing new views to my research. I also appreciate the positive attitudes, thoughts, and input that you both have provided me here at UF and towards my future career.

A huge debt of gratitude is given to Dr. Sally Johnson for exposing me to muscle biology and allowing me to come into her laboratory and turn it upside down. I initially thought that I would never learn the techniques that I know now, but you taught me that I am capable of learning and accomplishing anything I want. I appreciate the amount of time and money you spent on this project, and know that I could not have done this without you. Thank you for pushing me to publish, teaching me how to handle the criticisms (even when they were not right), and always being so encouraging. I know you will miss all my great sentences, but if you ever feel the need to read them, just give me a call.

I would next like to thank all my friends and coworkers here at the University of Florida. I owe a huge thank you to all the lab members in Dr. Sally Johnson's laboratory: Sarah Reed, Ju Li, and Dane Winner. Thank you for answering all my questions and teaching me the techniques necessary to complete my work. A world of thanks goes to Sarah Reed, because without you I would still be trying to figure out calculations, and your friendship has been very valuable to me. Thank you for being my best friend in the lab and always listening to me when I was complaining about something. I wish nothing but the best for you and your husband. Thank you to the Meat's Laboratory Crew: Larry Eubanks, Tommy Estevez, Byron Davis, Ryan Dijkhuis, Frank Robbins, and all the student workers. Larry, without you I could have never had roasts instead of steaks (and the associated headaches that came with those roasts) for my last study. Seriously, thank you for all your help, encouragement, and for being my Gainesville dad. I also learned a great deal of what I know now from you. Ryan, thank you for all your help and the expertise you provided when we were conducting my research. It was a real pleasure working with you and please know that my last study would not have gone as smoothly without you. We had some great times in the office, and on the road.

I would like to also extend many thanks to past graduates of the University of Florida Department of Animal Sciences, including Dr. Nathan Krueger, Dr. Jeff Rhone, and Dr. Alex Stelzleni. Without your advice and encouragement, I could have never made it through school. Jeff, thanks for watching all those sporting events with me and always giving me words of encouragement. Alex, thank you for all of your advice and help with my study. I really do appreciate all the faith you have in me as a fellow colleague. Thank you all for being my friends.

Finally, I would like to thank both my Texas and Florida families. Because I come from a big Mexican family I will not name you all. Thank you for the encouragement, Joe, Becca, and Dad. Cheeto and Livie, thank you for reminding me that I'm not ready for kids. Mom, I love you and please know that this accomplishment is as much yours as it is mine. Wright family, thank you for all the love and support, and for making me feel a part of your family. Mrs. Wright, please know that everything I accomplish in life, I owe to you. Bunny and Mary, thank you for being my Florida family. I know at times I drive you two crazy, but please know that I do love you both for allowing me into your family. Last, but certainly not least, all the love in my heart goes to Sara. I really wish I could express how I feel about you, but there is not enough room for it here. I want to thank you for not only helping me complete all my studies and aiding me in writing my manuscripts, but for providing the most loving environment anyone could ever ask for. I was pretty lonely in Florida before I met you, but all that went away when I met you. You set up a wonderful home that I always knew I could go home to at the end of the day, and that made this experience a thousand times more enjoyable. I look forward to the life you, me, Bexar, and Astro have ahead of us, sandy bed and all. I know that you are going to be a wonderful vet and a great mother.

To end, I just want to let everyone know that I am not exactly sure where my career will take me or how successful I will be. But all I really need in life to make me happy is to know that each and every one of you all is proud of me.

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December 2008

Chair: D. Dwain Johnson
Major: Animal Sciences

The ability of ractopamine-hydrochloride (**RAC**) to affect muscle fiber morphometrics, satellite cell population, and shelf-life properties of various muscles of the beef carcass was explored over three studies. In the first two studies, RAC was supplemented to cull cows during the final 28-35 days of feeding and differentially affected muscles of these animals. In the first study, RAC increased ($P < 0.05$) the cross-sectional area (**CSA**) and diameter of type I muscle fibers in the *Longissimus dorsi* without affecting type IIA fibers. In the second study, RAC supplementation increased the CSA of type I fibers of the *Infraspinatus* ($P < 0.05$) and *Vastus lateralis* ($P < 0.15$), but did not affect ($P > 0.05$) the *Longissimus dorsi* or *Semimembranosus*. Of these four muscles, RAC increased ($P < 0.05$) the CSA of type IIA fibers in only the *Infraspinatus* and *Semimembranosus*. In both studies, RAC supplementation altered ($P < 0.05$) the muscle fiber isoform distribution, but did not increase ($P > 0.05$) the number of satellite cells or fiber associated nuclei counted. This indicates that all growth observed in type I or type IIA fibers happened independent of satellite cell incorporation into the muscle fiber.

In the third study, RAC supplemented to steers did not affect ($P > 0.05$) the CSA of either fiber isoforms nor the muscle weights or dimensions of muscles of the round and loin. The

muscle fiber isoform distribution of all muscles, except the *Semimembranosus*, was changed ($P < 0.05$). Ractopamine supplementation did not affect ($P > 0.05$) objective measures of color or nitric oxide metmyoglobin reducing ability during a five day simulated retail display. Ractopamine supplementation increased ($P < 0.05$) the amount of surface discoloration on steaks from the *Rectus femoris*, *Semimembranosus*, and *Vastus lateralis* during the final days of display when evaluated by trained panelists. Results from all three studies indicate that RAC supplementation to both cull cows and steers has a limited ability to increase muscle fiber CSA, but can affect surface discoloration during retail display.

CHAPTER 1
INTRODUCTION

For over 25 years, researchers have examined the use of β-adrenergic agonists (**BAA**) to alter growth rate and body composition of livestock. Ricks et al. (1984a) and Beermann et al. (1985) were two of the first researchers to report the advantages associated with BAA supplementation to livestock. Structurally, BAAs are similar to norepinephrine and epinephrine, which are natural catecholamines (Mills, 2002). The physiological activity of a BAA depends on its absorption, metabolism, and elimination rates. In addition, the distribution of the BAA to target tissues also affects a BAA's physiological activity (Smith, 1998). β-adrenergic agonists elicit their actions by binding to one or more receptors identified as β_1, β_2, and β_3 receptors. These receptors are located on the membranes of most mammalian cells. Their distribution varies between tissues and species, and can also vary within a given tissue between species (Mersmann, 1998). An agonist binds to a receptor at three points on the molecule: a β-hydroxyl group, an aliphatic nitrogen, and an aromatic ring (Smith, 1998). Omissions or substitutions at these points results in agonist differences in receptor binding or activity (Ruffolo, 1991).

Because these agonists alter growth by redirecting nutrients from adipose tissue deposition to skeletal muscle accretion, BAAs are known as repartitioning agents (Ricks et al., 1984a). Beermann et al. (1985) suggested using BAAs as a means to reduce the cost of producing meat animals by reducing feed grain input costs. Smith (1998) identified up to 15 BAAs used in livestock production. Of those 15, clenbuterol, salbutamol, cimaterol, ractopamine, and $L_{644,969}$ are commonly used in research studies. Ractopamine-hydrochloride (**RAC**) and zilpaterol-hydrochloride are the only two BAAs approved for use in the United States. Ractopamine was developed by Elanco and approved for use in pigs by the Federal Drug Administration in 1999. This product is currently sold under the trade name Paylean. Four years later, in 2003, Elanco

developed and received approval for the use of RAC in beef cattle. This product is sold under the trade name Optaflexx and is approved for use in beef cattle during the final 28-42 days of feeding prior to harvest.

Historically, cows are culled in the fall for various reasons that include infertility, old age, and poor reproductive performance. Income from culling practices can account for 15-20 percent of an operation's animal proceeds (Feuz, 1999). Little consideration is given to feeding cull cows as a means to improve carcass quality. However, when market conditions are favorable, feeding cull cows is an attractive option. Matulis et al. (1987) reported that as time on feed is increased, cull cow carcass weight, fat thickness, marbling and quality grade are improved. Carcasses from fed cull cows demonstrate brighter, more youthful lean color and larger ribeye areas (Miller et al., 1987). The increased nutrients and energy intake causes a marked increase in fat deposition with little improvement in muscle accretion during the final days of finishing (Brown and Johnson, 1991; Boleman et al., 1996; Cranwell et al., 1996a). Therefore, RAC supplementation may remedy this by directing nutrients away from fat deposition and toward muscle accretion.

In swine, RAC supplementation profoundly increases lean deposition, while reducing the fat of the carcass (Yen et al., 1990; Dunshea et al., 1993). In young beef cattle, RAC modestly increases muscling as indicated by increases in ribeye area, but rarely affects fat deposition (Walker et al., 2006; Winterholler et al., 2007). Postnatal muscle growth is accomplished by muscle fiber hypertrophy. Hypertrophy involves an increase in fiber cross-sectional area via protein accretion. In order to maintain the increased protein synthesis demands of the growing fiber, nuclei are added to maintain an appropriate myonuclear domain (Kim and Sainz, 1992). Satellite cells proliferate, differentiate, and fuse with the muscle fibers to provide additional nuclei and DNA for the synthesis of protein (Aberle et al., 2001). In pigs, RAC causes muscle

growth by stimulating increases in type II fiber cross-sectional area without affecting type I fiber size (Aalhus et al., 1992). Beermann et al. (1987) and Kim et al. (1987) reported that DNA concentration per gram of protein was lower in beta-agonist supplemented lambs. Both groups concluded that muscle growth was due to a reduction in protein degradation and not satellite cell activity. However, a study has yet to measure the effect of RAC on actual satellite cell and fiber associated nuclei numbers. Therefore, the cellular mechanisms responsible for the increases in the cross-sectional area of the ribeye and other muscles of the beef carcass due to RAC supplementation are not well documented and require further investigation.

A common effect of BAA supplementation to livestock is muscle fiber isoform shifts. In pigs, RAC supplementation shifts muscle fiber myosin heavy chain isoforms toward more glycolytic isoforms (Aalhus et al., 1992; Depreux et al., 2002). Vestergarrd et al. (1994) reported that cimaterol increases the percentage of glycolytic fibers at the expense of oxidative fibers. With the shift toward glycolytic fibers, NADH content may be reduced and affect shelf-life. NADH is an integral part of the metmyoglobin reducing system that chemically reduces metmyoglobin to deoxymyoglobin (Mancini and Hunt, 2005). However, the effect of RAC supplementation on the myosin heavy chain isoform shift in beef cattle and the effects of this shift on shelf-life remain unexplored.

While numerous studies examine the effects of RAC on gross carcass characteristics of beef cattle, few have focused on the cellular events behind these effects. The objective of this dissertation is to: (1) examine the effect of RAC supplementation on muscle fiber morphometrics of various muscles throughout the beef carcass of young and old animals; (2) examine the effect of RAC supplementation on satellite cell and fiber associated nuclei populations; (3) examine the effect of RAC supplementation on muscle fiber myosin heavy chain isoform distribution of

various muscles throughout the beef carcass of both young and old animals; (4) determine if RAC induced shifts in myosin heavy chain isoform distribution affect the shelf-life of steaks during retail display.

CHAPTER 2
LITERATURE REVIEW

Beta-Adrenergic Receptors

Almost every cell type in the mammalian body possesses β-adrenergic receptors (**BAR).** In its plasma membrane BARs can vary in number and type. Receptors contain more than 400 amino acids in a continuous chain that form seven hydrophobic transmembrane domains, anchoring the receptor to the plasma membrane. The ligand binding site of the receptor is located within the seven transmembrane domain and this domain also interacts with the G_S protein. When an agonist binds to the seven-transmembrane G-coupled receptor, it activates intracellular adenylyl cyclase activity. The activation of adenylyl cyclase increases the intracellular levels of cyclic AMP. Cyclic AMP binds to the regulatory subunit of protein kinase A, releasing the catalytic subunit, which can phosphorylate numerous intracellular proteins. The phosphorylation of these proteins can either increase the transcription of a gene, as with skeletal muscle accretion, or limit enzymes, such as the enzymes responsible for long-chain fatty acid synthesis (Liggett and Raymond, 1993; Mersmann, 1998; Norman and Litwack, 1997).

Scientists have identified the three BAR subtypes in mammals as β_1, β_2, and β_3. Each BAR subtype exhibits a distinct RNA transcript size, protein size, and amino acid sequence. The three BAR subtypes maintain approximately 50% homology in amino acid sequence within a single species. Across species, individual BAR subtypes display 75% to 90% homology. Differences in sequence allow for variation in ligand affinity and receptor activation. Therefore, one ligand may have different affinities and a receptor activation capability for different BAR subtypes within a species. Similarly, ligand affinity and receptor activation capabilities vary across species for a specific BAR subtype. This leads to extensive diversity in the responses generated by ligands. Generally, BAR subtypes exhibit ligand selectivity for norepinephrine as $\beta_1 > \beta_2 > \beta_3$.

For epinephrine, β_1-receptor and β_2-receptor bind similarly. The β_2-receptor has a greater affinity for epinephrine than norepinepherine (Mersmann, 1998; Mills, 2002).

Because BAR subtypes generate diverse responses to different ligands, determining the distribution of these subtypes in mammalian tissues holds importance. Different species can have different BAR subtype concentrations distributed throughout their tissues. In swine, the β_1-receptor represents the most abundant BAR. This subtype comprises 80% and 60% of the BAR in adipose tissue and skeletal muscle, respectively. However, Sillence and Matthews (1994) suggest that the β_2-receptor comprises the majority of the BARs in beef cattle skeletal muscle and adipocytes. Van Liefde et al. (1994) suggest that cattle adipocyte BARs are composed of approximately 75% β_2-receptors and 25% β_1-receptors. Sissom et al. (2007) found that the expression of the β_2-receptor in skeletal muscle was 10-fold greater than the other two subtypes, indicating that the β_2-receptor is the most abundant receptor in bovine skeletal muscle. Mammalian tissue minimally expresses the β_3-receptor. This receptor is found mostly in adipose tissue, and only comprises approximately 10% of the BAR (Mills, 2002).

To avoid indefinite activation of BAR by ligands, the agonist can be removed or degraded, or the receptor may be inactivated by several mechanisms. These processes serve as safety mechanisms to prevent overstimulation of receptors during times of chronic exposure to agonists. The extent of desensitization of a receptor depends on the degree and duration of the receptor/agonist interaction (Johnson, 2006). These mechanisms of receptor inactivation include phosphorylation of the BAR by a kinase or complete removal of the BAR from the plasma membrane (Mersmann, 1998). Phosphorylation of the receptor by closely related G protein-coupled receptor kinases, such as PKA, serves as the principle mechanism of short-term agonist-promoted desensitization. Due to phosphorylation, β-arrestin binds and partially uncouples the

agonist-occupied form of the receptor from the G_s protein. Therefore, this limits receptor function by preventing continuation of the signal by the G_s protein. In addition, β-arrestins may bring other proteins into the receptor microenvironment. These proteins then act to metabolize cAMP and thus block the effects of receptor activation. Sequestration may also play a major role in short-term receptor regulation. This mechanism commonly occurs during periods of prolonged agonist exposure. During sequestration, receptors become internalized, which results in a loss of receptors from the cell surface. Sequestration takes longer to reverse because dephosphorylation of the receptor must occur while the receptor is internalized (Johnson, 2006).

The body can also regulate BARs by the complete removal of receptors from the cellular membrane. This process is termed downregulation and can differ markedly from cell to cell and tissue to tissue. Downregulation activates the process of receptor degradation by ubiquitination of the BAR through an E3 ligase. In order to restore original levels of membrane BAR, cells and tissues require transcription and posttranslational conversion of BAR mRNA to protein. In addition to this process, downregulation also can occur by modulation of BAR gene expression through the cAMP pathway (Johnson, 2006). Several current studies report a variety of effects of β-adrenergic agonists (**BAA**) on mRNA expression of the three BAR subtypes.

The earliest studies reporting BAA induced downregulation of BARs involve rats (Sillence et al., 1991). Kim et al. (1992) supplemented 10 ppm of cimaterol for up to 28 days and found that BAR binding sites in the plantaris muscle were reduced by 26.8, 42.2, 37.7, and 37.8% at 3, 7, 14, and 28 days, respectively. Researchers concluded that the decrease in growth promoting effects seen in the study was due to alterations in the number of BAR binding sites. In a recent study involving Holstein steers, Walker et al. (2007) reported that supplementing 200 mg of ractopamine-HCl (**RAC**) per day during the final 28 days of a 56 day feeding trial decreased

mRNA expression of both β_1- and β_2- receptors in the *Longissimus dorsi* (**LD**). Sissom et al. (2007) reported that expression of the β_3-receptor was not affected by RAC alone, but decreased when 200 mg/d of RAC was combined with a Revalor-200 implant. Gunawan et al. (2007) supplemented RAC to swine and reported that β_2-receptor expression decreased significantly by week 2 of supplementation. Since RAC supplementation tends to shift muscle fiber type from a slow to fast isoform, the researchers suggested that losses in β-adrenergic receptor expression were due to the loss of slow fibers, which have greater expression of β-adrenergic receptors.

In contrast to these findings, other results suggest that RAC has the ability to increase BAR expression or have no effect on BAR expression. In beef cattle, Winterholler et al. (2007) and Sissom et al. (2007) reported that RAC supplementation did not affect the expression of β_1- and β_3-receptors. The lack of effect on β-adrenergic receptor expression of both receptor subtypes also was reported in pigs (Gunawan et al., 2007; Mills, 2002; Spurlock et al., 1994). In contrast, Winterholler et al. (2007) fed 200 mg/steer of RAC daily for the final 28 days of feeding and reported that the expression of β_2- receptors tended to increase. The authors suggested the increase in expression was due to weak binding of RAC to β_2- receptors, which triggered the synthesis of new β_2- receptor protein. The authors also suggested that the synthesis of new protein could be due to muscle attempting to overcome the down regulation that occurs due to chronic administration of RAC. In agreement with this study, Sissom et al. (2007) examined the effect of 200 $mg \cdot hd^{-1} \cdot d^{-1}$ of RAC on heifers treated with various implant strategies, with similar results. Researchers found that RAC supplementation combined with a Revalor-IH/Finaplix-H implant strategy increased mRNA levels of β_2-receptors, and increased mRNA levels of β_3-receptor. The authors hypothesized that the increases in expression of BAR two and three may be due to manipulation of the muscle fiber isoforms elicited by RAC supplementation.

Effect of Beta-Adrenergic Agonists on Muscle Fiber Isoforms

Adult skeletal muscle traditionally consists of four myosin heavy chain isoforms identified as types I, IIA, IIX, and IIB. Although these isoform designations are based on inherent ATPase activity, muscle fibers may also be called by other nomenclature based on the assay used to identify them. Each isoform exhibits subtle differences in amino acid sequences, which creates differences in protein functionality. Type I and IIA fibers are commonly referred to as red muscle fibers, while type IIX and IIB fibers are referred to as white fibers. The differences between red and white fibers lie in their content of the oxygen binding protein myoglobin. Red fibers contain more myoglobin due to their use of oxidative metabolism. White fibers operate on glycolytic metabolism and contain less myoglobin than red fibers. Because red and white fibers differ in the type of metabolism they utilize, they contain different organelles and energy sources in their cellular matrix. Since red fibers operate on oxidative metabolism, they have a greater number of larger mitochondria, a greater lipid content, and lower glycogen content, compared to white fibers. The speed of contraction once stimulated also differs between fiber types. Type I fibers contract slower than type II fibers, with type IIB fibers contracting the fastest. Each fiber type also fatigues at different rates, with type I being the least susceptible to fatigue, followed by IIA, and then IIX/IIB. In most mammalian species, type I and II fibers are randomly intermingled within muscle bundles, but the primary fiber type that comprises the muscle determines its functional ability (Aberle et al., 2001).

At birth, bovine muscle contains approximately 40-50% type IIB fibers, 35-45% type IIA fibers, and 10-20% type I fibers. The percentage of each fiber type isoform varies slightly between breeds and can be affected by age. After birth, the main fiber type shifts occur during the first two months of life. From birth until two months of age, type IIB fibers increase at the expense of type IIA fibers. After two months of age, the rate at which type IIA fibers convert to

type IIB fibers decreases dramatically (Wegner et al., 2000). Jurie et al. (1999) report that the conversion of fast oxidative fibers to fast glycolytic fibers can occur at up to 12 months of age, with the fiber type frequency remaining constant after 12 months. Wegner et al. (2000) found that the percentage of the type I fiber isoform remains constant or changes slightly from birth until two years of age. In rats, muscles contract slowly at birth and increase in contraction speed during the first few weeks of life, indicating a slow to fast fiber type transition (Kelly and Rubinstein, 1980).

The postnatal shifts of muscle fiber isoform reported above can be attributed to the altered functional demands of the muscles observed. The muscle fibers of beef cattle fed in confinement had a lower oxidative metabolic potential when compared to muscle fibers from beef animals raised in an extensive production system (Vestergaard et al., 2000). In addition to postnatal activity, other factors such as nutrition can play a role in fiber type shifting. During early postnatal development, under-nutrition delays the shift of type I to type II fibers (Ward and Stickland, 1993; White et al., 2000). When comparing feedlot steers to non-supplemented range steers, Beerwinkle et al. (1979) found that range steer muscle contained a larger proportion of type I fibers. These results were most likely due to the differing nutritional status of the two groups of cattle in the study, with the range cattle having a lower plane of nutrition.

Nutritional supplementation in the diet can also affect fiber type isoform shifts. As mentioned earlier, numerous studies report that supplementation with RAC causes fiber type shifts in skeletal muscle. This is important from a muscle growth and BAA efficiency standpoint. According to Aalhus et al. (1992), the response to BAA supplementation is dependant on the initial fiber type. Therefore, different muscles, breeds, and species would have different responses based on the initial fiber type composition. Numerous studies have

demonstrated different combinations of fiber type shifts. With BAA supplementation, various rat muscles (*Soleus*, *Diaphragm*, and *Extensor digitorum longus*) showed increases in the percentage of type IIA (Bricout et al., 2004; Rajab et al., 2000), type IIX (Bricout et al., 2004; Criswell et al., 1996; Maltin et al., 1986; Polla et al., 2001), or type IIB (Maltin et al., 1986; Polla et al., 2001) fibers at the expense of type I fibers.

Ractopamine-HCl supplementation to pigs increased the percentage of type IIB fibers at the expense of type IIA fibers in a variety of muscles. However, the percentage of type I fibers remained the same when evaluated by histological techniques (Aalhus et al., 1992) and ELISA (Depreux et al., 2002). To further investigate the effect of BAA on muscle fiber isoform distribution, Gunawan et al. (2007) examined the effect of RAC supplementation on mRNA expression of the four myosin heavy chain isoforms in pigs. In the study, pigs were fed RAC for eight different time periods and harvested. Following harvesting, mRNA expression was quantified using real time PCR analysis. In agreement with other pig data, this study indicated that type I fiber expression was unaffected by RAC administration. Type IIA expression decreased by 96 hours after administration and continued to fall for one week. By the end of the 4 week trial, type IIA mRNA expression returned to pre-supplementation levels. It appears that type IIB expression increased throughout the trial at the expense of type IIX expression, which decreased by week 2 and continued to fall until the end of the trial. Therefore, these findings support other data demonstrating that RAC causes increases in type IIB fibers at the expense of type IIA and IIX fibers.

Studies analyzing the effect of RAC and other BAA on ruminant muscle fiber distribution are not as extensive as in other species. Kim et al. (1987) reported that lambs fed 10 ppm of cimaterol for 8 weeks showed no difference in the proportion of type I and type II fibers in the

Semitendinosus or the *Longissimus dorsi*. In another study, Beermann et al. (1987) fed 10 ppm of cimaterol to lambs for seven and 12 weeks, finding mixed results based on muscle. In the *Longissimus dorsi* and *Semimembranosus*, supplementation did not affect the percentage of type I fibers. However, in the *Semitendinosus*, the percentage of type I fibers was slightly reduced. Examining beef cattle, Vestergaard et al. (1994), uncovered results similar to swine data. In the study, four pairs of monozygotic twins were fed either 0 or 0.06 mg of cimaterol per kilogram of live weight per day for 90 days. Data indicated that the percentage of IIB fibers increased due to a decrease in the percentage of type IIA fibers with supplementation. The percentage of type IIB fibers also increased due to a decrease in type I fibers. This contrasts swine data, in which BAA supplementation does not affect the percentage of type I fibers.

Effect of Beta-Adrenergic Agonists on Muscle Fiber Morphometrics

Muscle is comprised primarily of fibers which are gathered into groups of 20 to 40 muscle fibers known as primary muscle bundles. Variable numbers of primary muscle bundles are grouped to form secondary muscle bundles. Variable numbers of secondary muscle fiber bundles are then grouped, yielding the whole muscle. Because the building blocks of muscle consist of muscle fibers, muscle mass is mainly determined by the number and size of fibers in any given muscle. In muscle, growth is achieved through both hyperplasia and hypertrophy. Muscle growth accomplished through hyperplasia consists of an increase in muscle fiber number. Muscle growth through hyperplasia occurs primarily during postnatal development and to some extent for the first few months following birth. Hypertrophic growth is accomplished by enlargement of individual muscle fibers. Commonly, hypertrophy of muscle fibers occurs through increases in the cross-sectional area (**CSA**) or diameter of the fibers of that muscle. Therefore, since the number of muscle fibers present in a muscle is set at birth, postnatal muscle

growth is accomplished through muscle fiber hypertrophy (Aberle et al., 2001; te Pas et al., 2004).

The nutritional status of an animal can affect myosin heavy chain distribution, as well as the CSA and diameter of muscle fibers. The magnitude of the nutritional effect on fiber size is dependent on the developmental stage of the animal and the muscle type observed (White et al., 2000). Studies have shown that the type I fibers in older, under-nourished animals are minimally reduced in CSA while the type II fibers are greatly decreased in CSA (Everitt et al., 2002; Goldspink and Ward, 1979; Stickland et al., 1975). When comparing feedlot steers to non-supplemented range steers, Beerwinkle et al. (1979) found that the range steer muscle had smaller fiber diameters and a larger proportion of type I fibers. These results were most likely due to the difference in nutritional status between the two groups of cattle, with the range cattle having a lower plane of nutrition. More importantly, the decrease in fiber CSA and diameter resulting from poor nutrition can be reversed in mature animals (Goldspink and Ward, 1979).

While improved nutritional status increases muscle fiber CSA and diameter, numerous studies report that supplementation with BAAs also increase these parameters. Many studies involving the effects of BAA supplementation on muscle fiber hypertrophy focus on the supplementation of clenbuterol to rats. However, the fiber type isoform affected by supplementation is not consistent across studies. In rats, RAC supplementation generally causes increases in type II fiber CSA. Criswell et al. (1996) found that clenbuterol supplementation does not affect the CSA of type I fibers, but increases the CSA of type IIA fibers. In agreement, Bricout et al. (2004) reported that type IIA fiber CSA doubled in non-injured muscle, while type I fibers were not affected. However in the same study, injured muscle treated with clenbuterol showed an increase in the CSA of both type I and IIA fibers. This may indicate that the injury

status of muscle affects its response to BAA. Herrera et al. (2001) also reported that clenbuterol increased the CSA of type I and type II fibers by 30 and 13 percent, respectively, in the unweighted hindlimbs of 30 month old rats. The 30 month old rats and 12 month old rats responded to clenbuterol treatment differently. In 12 month old rats, type I fibers increased in CSA due to clenbuterol supplementation while type II fibers were unresponsive. This may indicate that an animal's age affects the responsiveness to BAA administration.

Polla et al. (2001) also noted an age effect on BAA responsiveness. Twenty-one day old rats were supplemented with clenbuterol for 28 days. The CSA of type I, IIA, and IIB fibers from four distinct muscles were analyzed. In the D*iaphragm* and S*uperficial tibialis anterior*, type IIB CSA increased. Type I and IIA fibers of the *Soleus* decreased in CSA. Researchers concluded that growth stimulation by clenbuterol administration is age dependent and only detectable in young adult rats. In addition, the researchers hypothesized that decreases in the CSA of both type I and IIA fibers was due to both hypotrophy and shift in fiber types between the two isoform types.

Rat research also has demonstrated that long term administration of BAA limits its effectiveness in increasing fiber CSA. Using male rats, Maltin et al. (1986) found that the growth promoting effects of clenbuterol were selective for muscle fiber isoform and decreased over time. Rats were fed clenbuterol for 4 or 21 days and the CSA of the muscle fibers from the *Soleus* and *Extensor digitorum* were analyzed. The *Extensor digitorum* responded to supplementation in a time dependent manner. After four days of treatment, the cross-sectional area of slow-oxidative and fast-oxidative-glycolytic fibers increased significantly. However, after 21 days of supplementation, the CSA of neither fiber isoform responded, indicating that the effects of supplementation last for a limited time period. In agreement, Zerman et al. (1988)

found that chronic administration of clenbuterol increased the CSA of type I fibers of the S*oleus* for up to 2 weeks, but had no effect thereafter.

While numerous rat studies examine the effects of BAA on muscle fiber CSA and diameter, few studies focus on other species. In an early study examining the effect of BAA on lambs, Beermann et al. (1987) reported that cimaterol increased type I and type II muscle fiber CSA in the S*emitendinosus* by 30.4 and 29.3 percent, respectively. However, Kim et al. (1987) found that only type II fiber CSA of the *Semitendinosus* and *Longissimus dorsi* increased by 50 percent when using the same dosage level. The inability of cimaterol to affect the type I fiber CSA in the study may have been due to a shorter supplementation period employed by the researchers. This may indicate that supplementation duration affects which fiber types respond to supplementation.

In the study by Beermann et al. (1987), researchers noted that BAAs are variably effective across muscles. Type I and II fiber CSA of the *Longissimus dorsi* increased by 13 and 15 percent, respectively. However, greater increases were observed in the *Semitendinosus*. Maltin et al. (1990) also noticed this trend when supplementing clenbuterol to Friesian bull calves. Mean general fiber area increased by 50 percent in the *Semimembranosus*, while the mean fiber area increased by 13 percent in the *Triceps*. Currently, research has not determined why muscles react differently to BAA supplementation. Some scientists hypothesize that the disparity between muscles is due to the fiber type composition and thus the receptor density and affinity differences between fiber types. According to Martin et al. (1989) type I fibers contain more β-receptors than type IIB fibers, but receptors on type IIB fibers have a higher affinity for β-agonists. Therefore, since the *Semitendinosus* has more type IIB fibers than the *Longissimus*

dorsi (Kirchofer et al., 2002), it displays a higher affinity for β-agonists and exhibits an increased response.

Two studies examining swine and cattle support this hypothesis. Swine were supplemented with RAC, and the red fiber CSA of the *Semimembranosus* and *Psoas major* were not significantly affected. However, the CSA of both intermediate and white fibers increased by 24.3 and 16.8 percent, respectively (Aalhus et al., 1992). In agreement, Maltin et al. (1990) found that clenbuterol fed cattle exhibited increased CSA of fast twitch glycolytic and fast twitch oxidative glycolytic fibers, with no effect on slow oxidative fiber CSA. Vestergaard et al. (1994) supplemented cimaterol to bulls and uncovered opposing results. The CSA of type I fibers of the *Longissimus dorsi* were significantly increased, while type IIA fibers were not significantly affected, but were reduced in CSA. In the same study, *Semitendinosus* type I and type IIB fibers were found to increase in CSA. Due to the lack of a large number of studies examining BAA supplementation to food producing animals, more research is needed to evaluate the effect of BAA on muscle fiber morphometrics.

Beta-Adrenergic Agonists Mechanism of Muscle Growth and the Effect on Tenderness

As mentioned earlier, muscle growth occurs through both hypertrophy and hyperplasia. If one were to put both into an equation explaining the mechanism of growth, it would read as: growth equals the number of muscle cells plus protein accretion minus protein degradation. Therefore, hypertrophy through myofibrillar protein accretion represents the balance between myofibrillar protein synthesis and myofibrillar protein degradation (Forsberg et al., 1989).

Initially, scientists studying BAAs found that BAAs divert nutrients away from adipose tissue towards muscle accretion. However, they did not know which part of the muscle growth equation was responsible for the increased lean accretion. Ricks et al. (1984a) and Beermann et al. (1985) first proposed that BAA supplementation in livestock caused muscle growth through

decreasing protein catabolism and/or increasing protein synthesis. Swine studies contributed the majority of data supporting acceleration of protein synthesis as the mechanism of muscle growth (Dunshea et al., 1993; Dunshea et al., 1998; Williams et al., 1994). Mersmann (1998) supports these findings, stating that different BAAs increase blood flow to certain regions of the body, thus increasing muscle accretion by the enhanced delivery of substrates and energy needed for protein synthesis.

However, other investigators examining rats and sheep found no effects on muscle protein synthesis in BAA treated subjects, and concluded that muscle growth was due to altered protein degradation (Bohorov et al., 1987; Reeds et al., 1986). Most of the early work that investigated the mechanism behind increased muscle hypertrophy focused on protein degradation rates. Beermann et al. (1987) and Kim et al. (1987) reported that DNA concentration per gram of protein was lower in clenbuterol supplemented lambs. Based on these results, both groups concluded that muscle growth was due to a reduction in protein degradation and occurred independent of satellite cell activity. Similar observations were reported for rats (Maltin et al., 1986), lambs (Bohorov et al., 1987), and poultry (Gwartney et al., 1992) fed clenbuterol.

Forsberg et al. (1989) conducted a premier study examining the effects of BAAs on calcium-dependent proteases which regulate muscle protein degradation. In their study, the researchers supplemented rabbits with cimaterol for 35 days before harvest. Results indicated that cimaterol did not affect cathepsin B, cathepsin D, or neutral serine. However, cimaterol did reduce μ- and m-calpain by 58 and 57%, respectively. In addition, cimaterol reduced calpastatin activity by 52%. Wang and Beermann (1988) similarly found up to a 70% reduction in μ-calpain activity in lambs supplemented cimaterol. Therefore, the researchers concluded that hypertrophy

induced by cimaterol supplementation may involve an alteration of the calcium-dependent proteolysis system.

In disagreement with the previously mentioned data, other studies report increases in calpastatin activity due to BAA supplementation. Higgins et al. (1988) reported that clenbuterol increased calpastatin activity in sheep. Two other studies involving the supplementation of $L_{644,969}$ to lambs also found increases in calpastatin activity. The first study reported that at 0 days postmortem, calpastatin activity in the LD of treated lambs was 74% higher, while at four days postmortem, calpastatin activity was 430% higher than controls. Increased calpastatin activity resulted in lower extractable calpain I and the appearance of degraded myofibrillar proteins detected on SDS-PAGE gels for the treated animals (Kretchmar et al., 1990). In the second study, BAA supplementation increased calpastatin activity by 62.8% and 227.2% on day 0 and 7 postmortem, respectively. In disagreement with Kretchmar et al. (1990), (Koohmaraie et al., 1991) reported BAA supplementation increased μ- and m-calpain activity at 7 days postmortem. However, despite increased calpain activity, SDS-PAGE gel electrophoresis revealed that BAA administration slowed the appearance of myofibrillar proteins associated with postmortem proteolysis. These findings led the researchers to conclude that reduced muscle protein degradation plays a role in muscle protein accretion due to BAA supplementation.

Bardsley et al. (1992) conducted a series of experiments involving the supplementation of BAA to various species (cattle, poultry, rats, and lambs) and found that BAAs affected the calcium activated proteolytic system differently in each species. Trials indicated that cattle were more responsive to BAA treatment than the other three species. Cimaterol included at low levels in the diet of beef cattle for 9 or 16 weeks increased *Longissimus dorsi* calpastatin expression by 99 and 76%, respectively. The next most responsive species was sheep, which displayed a

maximum of 39% increase in calpastatin activity. Because all species do not respond similarly to cimaterol supplementation, Bardsley et al. (1992) suggested that one must consider the type of agonist, dosage, and duration of supplementation to achieve maximum effect on the calcium proteolytic system.

Wheeler and Koohmaraie (1992) conducted a study supplementing $L_{644,969}$ to cattle fed a high concentrate diet for 6 weeks prior to harvest. A 27% decrease in the fractional degradation rate of skeletal muscle myofibrillar protein was reported. At harvest, BAA administration had no effect on μ- and m-calpain activity, but on days 0 and 7 postmortem calpastatin activity increased by 60 and 348% percent, respectively. The researchers concluded that BAA induced hypertrophy resulted from reduced proteolytic activity because of increased calpastatin activity. In agreement with this study, Geesink et al. (1993) and Garssen et al. (1995) reported that clenbuterol administered to veal calves significantly increased calpastatin activity by 37 and 67%, respectively. Geesink et al. (1993) also reported clenbuterol tended to decrease μ-calpain by 34% and electrophoresis analysis indicated that clenbuterol administration reduced postmortem proteolysis by slowing the appearance of the 30 kDa myofibrillar degradation band. Therefore, these findings indicate that BAAs stimulate muscle hypertrophy by altering calcium dependent protease activity, more specifically, calpastatin.

Because BAAs stimulate muscle hypertrophy by decreasing muscle protein degradation through increased calpastatin activity, a toughening effect on meat tenderness occurs. In skeletal muscle, calpastatin acts as the endogenous inhibitor of μ- and m-calpain. In living muscle, μ- and m-calpain degrade muscle protein to aid muscle protein turnover. These proteases require calcium for activation, and once activated postmortem, these proteases are responsible for tenderization (Aberle et al., 2001). In discussing the importance of the calpain proteolytic

system, Koohmaraie and Geesink (2006) identified the BAA effect on calpastatin as the reason that meat from BAA treated animals becomes tougher.

The first studies to report decreases in tenderness due to BAA supplementation involved lambs. In these studies, lambs were supplemented 4 ppm of the BAA $L_{644,969}$ for 6 weeks prior to harvest. In the first study, treated lambs had 111 and 108% higher Warner-Bratzler shear force (**WBS**) values on day 3 and 6 postmortem, respectively, when compared to controls (Kretchmar et al., 1990). The second study reported that BAA administration increased WBS values for treated lambs by approximately 10, 48, and 70% on days 1, 7, and 14, respectively. Over the entire 14-day storage period, WBS values fell by 52.6% for control animals and by 18.3% for treated animals. Myofibril fragmentation index (MFI), a measure highly correlated with tenderness, also indicated that BAA-fed animals produced less tender meat. Over the 14-day aging period, their MFI increased by only 3.3%, while control animals' MFI increased by 42.3% (Koohmaraie et al., 1991).

Studies involving beef cattle report results similar to sheep data with respect to tenderness. Steaks from beef cattle fed the BAA $L_{644,969}$ for 6 weeks prior to harvest had WBS values that were greater than the controls on day 7 and 14 postmortem. In addition, WBS values for treated animals did not change during the entire 14 day aging period, indicating a lack of postmortem tenderization due to treatment. Myofibril fragmentation index for the study also indicated that control animals had more postmortem proteolysis than the treated animals throughout the 14 day aging period, which contributed to the greater WBS values reported (Wheeler and Koohmaraie, 1992).

Geesink et al. (1993) examined the effect of the BAA clenbuterol on the tenderness of four different muscles (*Longissimus, Semimembranosus, Triceps brachii, and Psoas major*) of veal

calves and found that multiple muscles are affected by supplementation. However, the results indicate that not all muscles are affected equally. Steaks from some treated muscles were tougher on day 1 or 7, while other muscles were unaffected. In a related study involving veal calves, Garssen et al. (1995) examined the effects of clenbuterol and salbutamol on *Longissimus lumborum* tenderness. Warner Bratzler shear force values for treated animals were higher on days 2, 5, and 10 postmortem. When compared to the control group, the salbutamol group produced the largest increase in WBS, which further indicates that various BAAs affect tenderness differently.

In 2007, the FDA approved a new BAA for use in beef cattle. Intervet sells this BAA, Zilpaterol-HCl, which acts through the β_2-receptor and seems to be more potent than RAC. Because this compound is new, limited literature exists documenting its effect on beef tenderness. However, it appears that this compound greatly affects tenderness. When fed to steers, zilpaterol increased shear values by 16 and 6% compared to control and RAC supplemented steers, respectively (Avendano-Reyes et al., 2006). More importantly, zilpaterol increased shear values of supplemented steaks to an average of 5.11 kg, which is nearly tough, as defined by Boleman et al. (1997) and Miller et al. (2001).

Since Optaflexx (ractopamine-HCl) gained FDA approval for beef cattle in 2003, research on the effect of RAC on beef tenderness has progressed slowly. The beef industry is justifiably concerned about this issue, given the literature involving other BAAs. For many years, Elanco, the manufacturer of the commercial form of RAC provided the only data on the effect of RAC on beef tenderness. These researchers used both objective and subjective measures to evaluate four concentration levels of RAC (0,100, 200, and 300 $mg \cdot hd^{-1} \cdot d^{-1}$) on strip loin steak tenderness. Both methods indicated that RAC fed at 100 and 200 $mg \cdot hd^{-1} \cdot d^{-1}$did not affect tenderness.

Warner-Bratzler shear force values of steaks from the 300 mg·hd^{-1}·d^{-1}group were 0.4 kg greater than control steaks. However, researchers concluded that this finding was not a major issue because these values were below 3.95 kg, which is considered acceptably tender to consumers. In addition, researchers stated that a 0.45 to 0.91 lb change in shear force is required for consumer recognition of a change. Trained sensory panelists found that Ractopamine fed at all levels did not affect juiciness, flavor, or the incidence of off-flavors. However, tenderness scores mirrored the findings of the WBS data. Compared to controls, initial and sustained tenderness scores were lower for the 300 mg·hd^{-1}·d^{-1}steaks. Once again, researchers stated that changes in tenderness detected by the panelists would not be detected by consumers and should not be a concern (Schroeder et al., 2004a).

Since these results were published, numerous university studies have concurred with the Elanco data. The first independent study that evaluated the effect of RAC on beef tenderness found that RAC fed at 300 mg·hd^{-1}·d^{-1} during the final 33 days of feeding significantly decreased tenderness by approximately 0.6 kg. The researchers attributed the decrease in tenderness to BAA's ability to reduced protein degradation and proteolytic activity, decreased collagen solubility, and changed fiber type distribution within the muscle. In agreement with Elanco, these researchers concluded that the RAC induced decrease in tenderness was a non-issue because the steaks were well within the acceptable range of shear force tenderness values (Avendano- Reyes et al., 2006). Possibly due to these findings, most university conducted studies have fed RAC at a rate of 200 mg·hd^{-1}·d^{-1}. Of these studies, two have evaluated the effect of RAC on tenderness. In agreement with Schroeder et al. (2004a), RAC supplementation at this level did not affect tenderness. Researchers attributed this lack of effect to a lack of growth in

the LD muscle. They hypothesized that the absence of growth did not signal an alteration in protein degradation and postmortem proteolytic activity (Quinn et al., 2008).

However, RAC supplemented at 200 $mg \cdot hd^{-1} \cdot d^{-1}$ for the final 28 days of feeding to British, Continental crossbred, and Brahman crossbred steers decreased longissimus muscle tenderness (Gruber et al., 2008). Daily RAC supplementation increased WBS and slice shear force by 0.38 and 1.4 kg, respectively. Ractopamine supplementation increased the WBS of Brahman crossbred steers more than the Continental crossbred and British steers. To counteract the toughening effect of RAC supplementation, steaks were aged for 3, 7, 14, and 21 days postmortem. Aging steaks did reduce WBS values, but did not completely reduce the RAC toughening effect. Ractopamine supplementation also affected trained sensory panelist scores. Trained sensory panelists found that RAC supplemented steaks were less tender and juicy, and tended to have reduced beef flavor. Researchers noted that because RAC is a β_1-agonist and this class of agonist causes muscle growth primarily through an increase in protein synthesis, the decrease in tenderness detected by objective and subjective evaluations was not due to increased calpastatin activity. The researchers hinted that RAC possibly increased the CSA and diameter of muscle fibers, decreasing tenderness, but that this hypothesis required further examination (Gruber et al., 2008).

Dijkhuis et al. (2008) examined the effect of three levels of RAC (0, 100, 200, and 300 $mg \cdot hd^{-1} \cdot d^{-1}$) on the tenderness of selected muscles from cull cows. Results established that RAC does not affect the tenderness of muscles uniformly. The tenderness of the *Infraspinatus, Rectus femoris, Semimembranosus, and Triceps brachii lateral head* were affected by RAC supplementation. The tenderness of other muscles, such as the *Adductor*, *Gracilis*, *Longissimus thoracis*, *Teres major*, *Triceps brachii long head*, and *Vastus lateralis*, were unaffected by RAC.

However, the muscles affected by RAC did not respond similarly to supplementation. Ractopamine fed at each level significantly increased shear force in the *Infraspinatus*. Ractopamine fed at 100 mg·hd^{-1}·d^{-1}increased tenderness in the *Semimembranosus*. In the *Rectus femoris*, RAC fed at 200 mg·hd^{-1}·d^{-1}resulted in steaks that were less tender than RAC steaks fed at the 100 mg·hd^{-1}·d^{-1}dosage level. The same trend was seen in the *Triceps brachii lateral head*, except steaks from the 300 mg·hd^{-1}·d^{-1} level were more tender than 100 mg·hd^{-1}·d^{-1}steaks. Researchers hypothesized that the increases in tenderness with RAC supplementation may be due to increased protein turnover in the 200 and 300 mg·hd^{-1}·d^{-1} treatment groups, which diluted collagen cross-linking.

Effect of Beta-Adrenergic Agonists on Swine Growth Performance and Carcass Characteristics

The main advantage of feeding RAC, and most BAAs, is that they increase lean deposition while restricting fat deposition in various species. These mechanisms translate into carcass and meat quality effects, as well as animal production improvements (average daily gain [**ADG**], average daily feed intake [**ADFI**], and feed:gain ratio [**F/G**]). Studies involving RAC supplementation in swine can be broken into two subgroups: effects on growth performance and effects on carcass and fresh meat characteristics.

The three primary growth performance characteristics improved by RAC supplementation include ADG, F/G, and ADFI. Watkins et al. (1990) found that when finishing pigs were supplemented with 0, 2.5, 5, 10, 20 or 30 ppm of RAC, ADG and F/G were improved linearly with increasing RAC level. In this study, supplementing RAC over a range of 5 to 15 ppm improved ADG and F/G over controls by 8.3 and 9.9%, respectively. Orthogonal polynomials for this study revealed that the dosage producing the maximum response in ADG was between 14 ppm and 16 ppm. Crenshaw et al. (1987) also studied these levels of RAC

supplementation and found that as RAC was increased, ADFI showed a liner decrease. Sities et al. (1991) ran a similar study examining the effects of 5, 10 or 20 ppm of RAC supplementation to finishing swine and found a similar linear increase in ADG with increase in RAC dosage level.

Prince et al. (1987) analyzed the same range of RAC supplementation as Watkins et al. (1990) and only found a tendency for RAC to improve ADG and F/G at the 5, 10, 20 and 30 ppm levels. The diminished effect may be due to the inclusion of a five day withdrawal period or insufficient number of animals. Crenshaw et al. (1987) found that RAC did not improve ADG or F/G. In agreement with this study, Hancock et al. (1987) found that ADG was unaffected by these levels of RAC supplementation, however a quadratic response was detected for F/G.

Studies have demonstrated that RAC supplementation to pigs during the finishing phase has the ability to positively affect ADG and F/G ratios. The response is rapid, occurring in as little as 6 days, with a constant supplementation level of 5 ppm. These effects are maintained by feeding 5 ppm and 20 ppm of RAC for 34 days (Armstrong et al., 2004). The enhanced growth response of pigs to prolonged RAC supplementation initially rises rapidly, reaches a plateau, and then declines during the rest of the feeding period (Dunshea et al., 1993; Williams et al., 1994). This trend indicates that prolonged RAC supplementation can cause a fatigue in receptor and growth responses. Sainz et al. (1993) noticed that RAC supplementation also improved weekly and ADG during the first three weeks of supplementation and not thereafter. See et al. (2004) observed the effects of feeding RAC at increasing (step-up), decreasing (step-down), and constant levels on growth performance. Researchers concluded that the step-down method desensitized β-adrenergic receptors and caused a reduction in the overall response to RAC supplementation. The step-up RAC supplementation protocol enhanced growth performance

(ADG and F/G) above the step-down or constant supplementation methods. Therefore, fatigue to RAC supplementation can affect response to supplementation, but the phase of production (weight and age) can affect magnitude of response.

Older and heavier pigs experience a more pronounced stimulation in growth performance than young pigs (Sainz et al., 1993). This indicated that phase of growth when RAC is added to the diet affects how an animal responds to supplementation. In contrast to this study, Crome et al. (1996) compared the effect of RAC supplementation in two different weight groups of finishing pigs (light, 68 to 107 kg; heavy, 85 to 125 kg). Average daily gain was improved with no difference between groups. Average daily feed intake was improved by RAC supplementation and the heavier group experienced a greater decrease. The heavier group responded to RAC supplementation with a greater decrease in F/G than the lighter group.

Watkins et al. (1990) stated that the response of growth and carcass variables to RAC supplementation may depend on supplementation level as well as other factors, such as genetics and the dietary crude protein content of the diet. Genetic studies have investigated the effect of crossbreeding and RAC supplementation on growth performance. In a study by Uttaro et al. (1993), results indicated that when 20 ppm of RAC was supplemented during the finishing phase of crossbred Canadian swine, ADG was improved by 150g/d and F/G was decreased by 0.52 kg. Yen et al. (1991) conducted a study where contemporary crossbred swine and crossbred Meishan swine were supplemented with 20 ppm of RAC with improved ADG and F/G, with no effect on ADFI. In contrast to these studies, Gu et al. (1991) determined that when RAC was fed to five different genotypes of crossbred pigs, with different lean growth potentials, there was no effect on ADG, ADFI, or F/G. Numerous studies which supplemented RAC to contemporary pigs

revealed no effect on ADG (Hancock et al., 1987, Prince et al., 1983; Ott et al., 1989). Contradictory results may be due to the genetic capacity of various breeds to deposit lean.

Pigs more genetically predisposed to increased lean tissue deposition respond more favorably to RAC supplementation. Bark et al. (1992) compared low and high lean tissue genotypes and found that both genotypes maintained the same ADFI, however ADG was increased by 41% and F/G was decreased by 32% for the high lean tissue genotype, compared to the low lean tissue genotype. Stoller et al. (2003) reported that ADG of high lean pigs was greater than ADG of purebred Durocs and purebred Berkshire, when all lines were supplemented with 20 ppm of RAC. Yen et al. (1990) compared genetically obese and genetically lean pigs supplemented with 20 ppm of RAC. Average daily feed intake and F/G ratio of both genetic lines were improved by supplementation, but ADG was unaffected by treatment. Lean pigs supplemented with RAC had higher F/G ratios than obese pigs supplemented with RAC.

By contrast, Mimbs et al. (2005) found that during the first 7 days of supplementation, lean pigs fed RAC gained less than fat pigs fed RAC. Over the course of the entire study, RAC did not affect ADG between the two genotypes. In agreement, Mitchell et al. (1990) supplemented RAC to the diets of high lean deposition line of pigs fed 12 or 24% crude protein, and found no significant effect on ADG or F/G. These researchers determined that RAC improves growth by improving carcass characteristics, but has no effect on live performance. However, this result may have occurred due to the low number of animals in each group.

The extent of the improvement seen with supplementation is influenced by many factors, including the factors previously discussed. The nutrient content of the diet, including energy and crude protein fed during supplementation also affects the magnitude of the RAC response. Williams et al. (1994) compared four levels of dietary energy content and found that ADG and

F/G were improved by RAC supplementation with a linear increase in ADG with increased energy content. Dunshea et al. (1993) compared six levels of protein (8.5, 11.2, 14.0, 16.7, 19.5 and 22%) and found that ADG increased with dietary protein content in RAC supplemented animals. Similarly, F/G improved (decreased) with increased dietary protein content. Adeola et al. (1990) reported that pigs fed different dietary protein levels respond differently to RAC supplementation. Pigs fed a 17% protein diet demonstrated increased ADG and F/G, while pigs fed a 13 % protein diet demonstrated decreased ADG and F/G.

By contrast, Mitchell et al. (1991) reported that RAC did not affect ADG or F/G at dietary crude protein levels of 12, 18, and 18 % restricted. The authors noted that the lack of RAC effect on ADG was due to performance during the latter stages of the study, possibly because of receptor fatigue. Dunshea et al. (1998) found that RAC increased ADG and decreased F/G in both gilts and boars, but this was achieved independently of dietary energy intake. As demonstrated by the above studies, RAC supplementation has an effect on growth performance and this response is determined by the level of supplementation, genetics, and diet. Not only do these factors affect growth performance, but also carcass and fresh meat characteristics.

Ractopamine supplementation mainly affects pig carcasses via increased deposition of lean tissue and decreased deposition of adipose tissue. This effect is positively correlated with improved dressing percentage. Dressing percentage increases linearly with increasing levels of RAC (Hancock et al., 1987; Stites et al., 1990; Watkins et al., 1990). Supplementation of RAC at 2.5, 5, 10, 20, and 30 ppm levels increased dressing percentage and carcass lean percentage, and decreased carcass fat percentage (Crenshaw et al., 1987; Hancock et al., 1987; Prince et al., 1987; Watkins et al., 1990). See et al. (2004) reported an increase in percentage lean using step-

up, step-down, and constant feeding regimens. Ractopamine supplementation also increased estimated fat free muscle when fed at these levels (Watkins et al., 1990).

Loin eye area is also used to demonstrate increased lean deposition with RAC supplementation. Various studies feeding RAC at levels of 2.5, 5, 10, 20, and 30 ppm found that loin eye area increased linearly with increasing RAC supplementation level (Stites et al., 1991; Watkins et al., 1990) and increased overall when compared to non-supplemented pigs (Crenshaw et al., 1987; Hancock et al., 1987; See et al., 2004). By contrast, Sainz et al. (1993) did not find a difference between the loin eye area of non-supplemented pigs and pigs fed RAC at various rates (constant, step-up, step-down, and alternating).

Carcass fat content is reduced by RAC supplementation. Ractopamine fed at levels of 2.5, 5, 10, 20 and 30 ppm reduced the 10^{th} rib back fat depth when compared to controls (Crenshaw et al., 1987, Prince et al., 1987; Watkins et al., 1990; See et al., 2004). Watkins et al. (1990) also reported lower average back fat depths for RAC supplemented animals, while Hancock et al. (1987) found that RAC supplementation only tended to decrease average back fat depth. Again, Sainz et al. (1993) found that average back fat thickness was not affected by RAC supplementation. Leaf fat deposited in the body cavity was reduced in RAC fed pigs compared to controls (Prince et al., 1987; Watkins et al., 1990; See et al., 2004).

Stites et al. (1991) reported that pigs fed 0, 5, 10, and 20 ppm of RAC had heavier loins and hams than controls. The percent weight of trimmed ham and loin was increased by RAC supplementation, while there was no effect on the percentage of trimmed shoulder, belly or boneless ham. These differential effects of RAC could indicate that RAC affects distinct muscle groups differently.

Moreover, a minimum time on feed with RAC is required to observe an effect on carcass measures. Ractopamine did not improve hot carcass weights at any level of RAC supplementation (5, 10, or 20 ppm) at 6 days of supplementation. However, after 13 days of supplementation, all levels of RAC supplementation improved hot carcass weights. After 20 days of feeding, RAC only improved the hot carcass weights in pigs supplemented 10 and 20 ppm. When fed past 27 days, the 20 ppm level of supplementation had increased hot carcass weights when compared to the other levels of supplementation. Pigs supplemented 20 ppm of RAC for 6 and 13 days demonstrated an increase in dressing percentage. Ractopamine supplemented for 20, 27, and 34 days at 10 and 20 ppm resulted in increases in dressing percentage. Ractopamine supplementation did not affect loin eye area when fed for 6, 13, and 20 days. However, pigs supplemented with 10 and 20 ppm for 27 and 34 days possessed increased loin eye areas. Percentage of fat free lean was only increased by RAC supplementation at 10 and 20 ppm for 27 and 34 days. There was no effect on 10^{th} rib back fat depth at any level of RAC supplementation for any of the time periods (Armstrong et al., 2004).

As previously stated, the response of growth performance to RAC supplementation is not constant over time, which may be attributed to receptor desensitization (Dunshea et al., 1993; Williams et al., 1994). This trend is reflected in carcass characteristics. When comparing RAC step-up, step-down, and constant feeding programs, See et al. (2004) found an improvement in hot carcass weight and percent yield for pigs fed the RAC step-up and constant program. The step-down program was not different from controls. Weights of the boneless-trimmed shoulder and loin were increased when the RAC step-up and constant feeding regimen were followed, while the RAC step-down program resulted in no effects on these cuts. Sainz et al. (1993) found that dressing percentage was increased by the constant RAC supplementation method, RAC

supplementation during the final three weeks of feeding, and an alternative weekly RAC supplementation method. The authors concluded that attenuation in growth and carcass characteristics observed during prolonged supplementation can be prevented by feeding RAC during alternating weeks.

Unlike growth performance data, the magnitude of response to RAC supplementation in pig carcasses is not affected by genotype. Yen et al. (1991) reported that 20 ppm of RAC fed to three diverse genotypes during the final 52 days of feeding increased hot carcass weight and dressing percentage regardless of genotype. In agreement, Gu et al. (1991) found that dressing percentage was increased by 1.28 percent in pigs of five different genetic backgrounds. In addition, loin eye area was also increased by RAC supplementation. Stoller et al. (2003) also reported improved loin eye area by 1.4 cm^2 when feeding RAC, but did not find an effect of genetic line on this improvement. Uttaro et al. (1993) found that feeding 20 ppm of RAC to crossbred swine decreased 10^{th} rib back fat depth, and increased 10th rib lean depth, which resulted in an increase in percent lean yield, regardless of genetic background. Also in this study, the weight of trimmed shoulders from RAC supplemented animals was decreased due to less trim needing removal. Loin weights of supplemented pigs were increased, which also resulted in an increase in salable lean. Longissimus muscle area of supplemented pigs was greater than controls, due to 13% more lean and 12% less fat being present. Trimmed hams from RAC fed animals had increased weight and decreased fat content, while belly lean was increased and fat content decreased.

In contrast to these studies, Stoller et al. (2003) reported that supplementation of 10 ppm of RAC to pigs of different genetic background reduced 10^{th} rib back fat in the high lean genetic line only. Yen et al. (1991) also found that RAC increased the *Longissimus* muscle area of high

lean yield and medium lean yield genotypes, while having no effect on the low lean yield genotype. In the same study, it was also determined that the high lean yield genotype had more muscling than the medium lean yield genotype, which had more muscling than the low lean yield genotype. These two studies show that the pig's genetic capacity to deposit lean has an effect on carcass characteristics in response to RAC supplementation.

Yen et al. (1990) compared the effect of RAC on obese and lean lines of pigs and found that in both obese and lean lines, RAC was effective in reducing body fat deposition and increasing carcass leanness. Hot carcass weight and dressing percentage were increased by RAC supplementation, while loin eye area at the 10^{th} rib was increased. The predicted amount of muscle was increased by RAC and the weights of the untrimmed picnic and trimmed Boston butt, picnic, and shoulder also were increased. The weight of the untrimmed or trimmed ham was unaffected by RAC supplementation, but proximate analysis for the ham showed decreased fat content and increased crude protein and moisture contents.

In a study by Mimbs et al. (2005), pigs were separated phenotypically into fat and lean groups and supplemented with 10 ppm of RAC. Ultrasound carcass measurements revealed that during the first three weeks of the trial, RAC did not affect deposited back fat. However, after the fourth week of the trial, the RAC supplemented pigs had lower ultrasound back fat measures. During the trial, there was a significant phenotype x RAC interaction, which indicates that the phenotypes responded to supplementation differently. *Longissimus* muscle area measured by ultrasound also was increased also by RAC supplementation in both groups, but no interaction was reported.

Finally, Bark et al. (1992) supplemented 20 ppm of RAC to high and low lean tissue growth genotypes of pigs and found that RAC reduced backfat thickness in both genotypes by a

similar magnitude. Ractopamine supplementation increased *Longissimus* muscle area in both genotypes, with the high genotype experiencing a greater magnitude of increase. Pigs of the high genotype tended to show increased estimated muscle and increased dissected muscle weights. Ractopamine also decreased the amount of dissectible fatty tissue more in the high genotype than in the low genotype, and the high genotype had more muscle in the ham, belly and shoulder. Despite the fact that RAC had no effect on the intramuscular fat content of the longissimus muscle of either genotype, RAC decreased the intramuscular fat content of the triceps brachii of the low genotype, with no effect on the high genotype. Therefore, it was determined that RAC included in the diet of a high lean yielding genotype and a low lean yielding genotype increases muscle accretion and decreases fatty tissue accretion to a greater degree in the high lean yielding genotype.

Rapid and low lean growth lines of pigs were fed 12% or 24% crude protein supplemented with 20 ppm of RAC from 60 to 90 kg of weight. Tenth rib back fat was reduced by 25% in the low lean pigs supplemented RAC and fed 12% crude protein, 16% in the high lean pigs supplemented RAC and fed 12% crude protein, and 7% in the high lean pigs supplemented RAC and fed 24% crude protein (Mitchell et al., 1990). Overall, carcass lipid was reduced the most by RAC supplemented to the low lean pigs supplemented RAC and fed 12% crude protein, than low lean pigs supplemented RAC and fed 24% crude protein, high lean pigs supplemented RAC and fed 12% crude protein, and high lean pigs supplemented RAC and fed 24% crude protein. There was a Line x Diet x Treatment interaction for backfat, backfat growth, and dissectible fat growth. For carcass and muscle protein, the low lean pigs supplemented RAC showed increases in carcass protein growth by 60% while the high lean pigs supplemented RAC increased carcass protein growth by 15%. This trend was true for *Longissimus* muscle area where low lean pigs

supplemented RAC pigs responded more than high lean pigs supplemented RAC pigs. A Line x Diet x Treatment interaction was observed for *Longissimus* muscle area, which indicates that the genetic background of the pigs determines the magnitude of the response, but protein level of the diet is important.

As demonstrated by the previous study, dietary factors such as crude protein content can affect the magnitude of the response to RAC supplementation. Additional studies examined dietary energy effects on RAC response. In a study by Williams et al. (1994), dietary energy intake has an effect on the magnitude of carcass characteristic response to RAC supplementation. In this study, four energy intake levels were used in combination with 44.7 mg/d of RAC. Ractopamine reduced backfat, increased *Longissimus* muscle area and increased dressing percentage. For dressing percentage, there was a linear trend toward an increase in dressing percentage with increasing energy intake level for RAC supplemented pigs. There was a RAC x Energy interaction for backfat thickness, and fat depth was increased for pigs supplemented with RAC at lower energy levels. A linear relationship between increased energy intake and increased fat thickness for RAC supplemented pigs also was noticed. *Longissimus* muscle area also had an Energy x RAC interaction and areas were greater for pigs supplemented RAC at lower energy intakes. These interactions, therefore, indicate that RAC is able to produce maximum gains in lean at lower energy intakes.

Dunshea et al. (1998) studied the effects of feeding five different energy levels on carcass characteristics of RAC supplemented pigs and found that RAC did not have an effect on average backfat thickness and dressing percentage, but increased loin eye area independent of dietary energy content. Empty body protein and water deposition was increased by RAC supplementation, while empty body fat and fat:protein ratio decreased with RAC

supplementation. For fat deposition, there was no effect of RAC supplementation, but the protein:fat ratio was increased by RAC. All these measurements were affected independent of dietary energy content, but RAC supplementation did increase protein deposition. As dietary energy increased, the response to RAC supplementation also increased. Unlike energy content of the diet acting independent of RAC supplementation, Dunshea et al. (1993) looked at six levels of dietary protein along with 20 ppm of RAC supplementation and found that empty body protein deposition was increased by RAC supplementation at the higher dietary protein levels.

Mitchell et al. (1991) fed three different levels of dietary protein and energy and two different level of RAC supplementation and found that even though crude protein levels had an effect on backfat thickness, RAC supplementation did not have an effect. There was a reduction in the amount of carcass lipid present in the study, with pigs fed a 12% (3.68 Mcal of DE/kg) crude protein diet having a greater reduction than the 18% (3.52 Mcal of DE/kg) crude protein diet. Likewise, RAC supplementation reduced the rate of lipid deposition and once again the 12% crude protein diet had the greatest reduction. *Longissimus* muscle area also was increased by RAC supplementation with the 18% crude protein diet having the greatest increase compared to the 12% crude protein diet. For carcass protein, the 12% diet had more protein than the 18% diet, and the rate of protein accretion was greater for the 12% diet.

Adeola et al. (1990) supplemented 20 ppm of RAC in 13 and 17% crude protein diets and found that RAC reduced leaf fat, increased muscle depth, increased primal and subprimal weights, and promoted rate of protein deposition in the *Longissimus* and *Biceps femoris*, regardless of protein level in the diet. The authors concluded that RAC supplementation increases carcass lean at both high and low dietary protein levels. Carcass weights were the only

characteristic where crude protein affected the magnitude of response to RAC. Weights were lower for RAC pigs fed the 13% protein diet when compared to the 17% protein diet pigs.

Few reports documenting the effect of RAC supplementation on fresh meat characteristics exist. Researchers have determined that RAC supplementation does not affect color, texture, or marbling (Hancock et al., 1987; Stites et al., 1991; Watkins et al., 1990; Sainz et al., 1993). In contrast to these studies, Watkins et al. (1990) reported increases in color, firmness, and marbling when pigs were fed 5, 10, or 20 ppm of RAC. Armstrong et al. (2004) found no difference in marbling between supplemented and control pigs at 6, 13, or 20 days of supplementation. However, at 10 and 20 ppm of supplementation, marbling was decreased at days 27 and 34. Color scores were not affected by supplementation on days 6, 13, or 34, but were lower for pigs supplemented 20 ppm for 20 and 27 days. This same trend was observed for L* and a* values. However, b* values were unaffected by dietary RAC concentration and length. Therefore, marbling and meat color of RAC supplemented animals can be affected by RAC supplementation.

Effect of Beta-Adrenergic Agonists on Cattle Growth Performance and Carcass Characteristics

Because RAC was just recently approved for use in beef cattle, few publications exist evaluating its effects on beef cattle. Historically, clenbuterol was the main β-agonist studied in cattle (Miller et al., 1988; Ricks et al., 1984b). One of the earliest RAC studies involving cattle was conducted by Anderson et al. (1989). In this study, six levels of RAC (0, 10, 20, 40, 60, and 80 ppm) were supplemented at two different dietary protein levels (11 and 14%). There was no RAC x Diet interaction and average daily gain was improved at 80 ppm of supplementation, while F/G was decreased at 20, 40, 60, and 80 ppm of RAC supplementation. For carcass characteristics, yield grade was decreased by 20, 40, 60, and 80 ppm of RAC supplementation;

percent protein was increased by 40, 60, and 80 ppm of RAC supplementation; and percent fat was decreased by 60 and 80 ppm of RAC supplementation.

Commercially RAC was approved by the Food and Drug Administration (**FDA**) in 2003. Elanco sells this product under the trade name Optaflexx® for cattle fed in confinement during the last 28 to 42 days of feeding. The first data involving the use of Optaflexx in beef cattle was a summary of the results of live performance and carcass characteristics for Elanco's FDA registration trials. The data presented represented 10 research trials evaluating the effects of RAC on both steers and heifers. Ractopamine was fed at approximately 100, 200, and 300 $mg \bullet hd^{-1} \bullet d^{-1}$ for either 28 or 42 days prior to harvest. All data for the 10 trials were pooled and a total of 220 steers and 215 heifers were examined. Ractopamine did not affect feed intake in either steers or heifers. In steers, RAC improved ADG by 17.1, 19.6, and 25.7% in the 100, 200, and 300 $mg \bullet hd^{-1} \bullet d^{-1}$ treatments, respectively. This resulted in feed efficiency improvements of 13.6, 15.9, and 20.5%. However, RAC does not seem to act as efficiently in heifers as it does in steers. While still significantly different, heifers fed 100, 200, and 300 $mg \bullet hd^{-1} \bullet d^{-1}$ of RAC had improved ADG of 8.0, 17.5, and 20.4%, respectively. Feed efficiency was improved by the treatment levels by 6.9, 14.0, and 17.1%, respectively.

Any improvements in carcass characteristics due to RAC supplementation also indicated that RAC has more of an effect on steers than heifers. Of the carcass characteristics evaluated, RAC significantly affected hot carcass weight, dressing percentage, ribeye area, and yield grade. Ractopamine improved hot carcass weight by as much as 18.2 lbs in steers and 11.3 lbs in heifers. Dressing percent was improved by 0.3 and 0.4 percentage points in the 200 and 300 $mg \bullet hd^{-1} \bullet d^{-1}$ treatment groups of steers. The dressing percent of heifers was unaffected by RAC supplementation. Ribeye area, an indicator of muscling, improved by as much as half an inch in

both steers and heifers fed 300 mg•hd^{-1}•d^{-1}. Ractopamine supplementation tended to decrease the yield grade of steers fed 200 mg•hd^{-1}•d^{-1} and significantly decreased the yield grade of steers fed 300 mg•hd^{-1}•d^{-1}. However, in heifers RAC, only tended to decrease the yield grade in the 300 mg•hd^{-1}•d^{-1} treatment group.

Producers are often concerned about feeding BAAs, including RAC, because of their effects on fat deposition. Across all ten studies for both steers and heifers, all fat measurements including KPH, 12th rib fat thickness, and marbling were unaffected by RAC supplementation. In addition, quality grades of both steers and heifers were unaffected by RAC. Other meat quality parameters such as color, firmness, and texture of meat from steers were unaffected by RAC. In heifers, color was improved by RAC, while the other two meat quality parameters were unaffected (Schroeder et al., 2004a,b).

Following the release of this data, researchers from Elanco published the results of the 10 individual studies in a series of abstracts. In the first abstract, a dose titration study was conducted on feedlot steers supplemented 0, 10, 20, or 30 ppm of RAC. Growth performance data indicated that RAC did not change feed intake, but improved ADG, F/G, G/F, HCW, and dressing percentage (Schroeder et al., 2005a). For the same animals at the same supplementation levels, *Longissimus* muscle area was increased. Yield grade tended to be improved at the 20 ppm supplementation level and was significantly improved at the 30 ppm supplementation level. Twelfth rib fat thickness and KPH were unaffected by RAC as well as muscle color, firmness, and texture (Schroeder et al., 2005b). Protein content also was increased at the 20 and 30 ppm supplementation levels (Schroeder et al., 2005c).

Schroeder et al. (2005d) analyzed these same levels of RAC supplementation fed to feedlot heifers and determined that feed intake was unaffected. However, RAC supplementation

improved ADG and G/F, and decreased F/G. Hot carcass weight was increased in heifers fed 20 and 30 ppm of RAC, while dressing percentage was unaffected by RAC supplementation. *Longissimus* muscle area was the largest for heifers fed 30 ppm of RAC and yield grade was improved by 30 ppm of RAC. Muscle color was improved with RAC supplementation. Twelfth rib fat thickness, KPH, muscle firmness, and muscle texture were unaffected by RAC supplementation (Schroeder et al., 2005e). The carcass composition of these heifers indicated that carcass protein and moisture were increased by 30 ppm of RAC supplementation (Schroeder et al., 2005f).

Laudert et al. (2005a) conducted a large pen study to examine the effects of RAC supplementation (0, 100, and 200 $mg \cdot hd^{-1} \cdot d^{-1}$) during the final 28 to 32 days of finishing in a feedlot setting. Ractopamine did not increase dry matter intake, but improved ADG, F/G, and G/F. These same feedlot finishing steers had increased dressing percentages when supplemented with RAC. Carcass dressing percentage was higher for the 200 $mg \cdot hd^{-1} \cdot d^{-1}$ supplemented group compared to the 100 $mg \cdot hd^{-1} \cdot d^{-1}$ supplemented group. Loin muscle area was increased with increased supplementation level of RAC, while marbling score was unaffected by supplementation. Finally, 12^{th} rib fat thickness, KPH, yield grade, and carcass maturity were unaffected by RAC supplementation (Laudert et al., 2005b).

Supplementation (0, 200, or 300 $mg \cdot hd^{-1} \cdot d^{-1}$) to calf-fed Holstein steers did not affect ADFI, while ADG and F/G were improved by supplementation (Vogel et al., 2005a). Carcasses from the supplemented steers were heavier than the controls. Longissimus muscle area was increased by supplementation, and yield grade was decreased by RAC supplementation. Marbling scores were decreased for steers fed the 200 $mg \cdot hd^{-1} \cdot d^{-1}$ of RAC, while the scores for

the 300 mg·hd^{-1}·d^{-1} supplementation were not affected. Feeding RAC did not affect KPH, carcass maturity, or the incidence of dark cutting (Vogel et al., 2005b).

Beginning in 2006, university sponsored research studies began to appear in the literature with most studies reporting similar results as the Elanco data. Walker et al. (2006) evaluated the effects of RAC (200 mg•hd^{-1}•d^{-1} for 28 days) and protein source (688, 761, and 808 g/d) on the growth performance and carcass characteristics of heifers. Feed efficiency was improved by RAC by 17%, while ADG increased by 18%. However, increasing metabolizable protein did not improve these growth performance measurements in RAC fed animals. Modest improvements in some carcass characteristics were reported. Final body weights of RAC supplemented animals were increased by 8.3 kg and HCW was increased by 6.9 kg. As was found with growth performance parameters, increasing metabolizable protein did not improve HCW or final body weight. Other carcass characteristics including dressing percentage, ribeye area, 12th rib fat, yield grade, and marbling score were unaffected by RAC supplementation or protein source. The authors concluded that while RAC improves some growth performance and carcass characteristics, the level of metabolizable protein does not need to be adjusted.

During 2007, numerous studies were published evaluating the effects of different management strategies and RAC supplementation on growth performance and carcass characteristics. Initially, researchers studied the effect of the age of beef cattle on the response to RAC. The goal of the study was to determine if differences in age affect the ability of a BAA to repartition nutrients. Yearling steers were serial harvested at 150, 171, or 192 days on feed. Within each harvest group, steers were fed 0 or 200 mg•hd^{-1}•d^{-1} of RAC for the last 28 days of feeding. The inclusion of RAC in the diet increased ADG and G:F ratio by 4.6 and 3.8%, respectively. During the last 28 days of feeding, RAC increased the dry matter intake compared

to controls by 3.5%. Ractopamine tended to increase ribeye area by 1.74 cm^2 and significantly increased HCW by 8 kg. All other carcass characteristics were unaffected by the inclusion of RAC in the diet. The authors reported no RAC × days on feed interaction, which indicates that RAC improves the aforementioned characteristics regardless of feeding length (Winterholler et al., 2007).

The influence of biological type on the RAC response was examined next. Steers of British, Continental, and Brahman biological type were fed either 0 or 200 $mg \bullet hd^{-1} \bullet d^{-1}$ for the final 28 days of feeding. Mirroring previous studies, RAC increased ADG and G:F ratio and did not affect dry matter intake. The only carcass characteristics significantly improved by RAC were HCW and ribeye area, which were increased by 2 and 4%, respectively. Ractopamine tended to lower marbling, but marbling was not lowered enough to affect the distribution of quality grade. However, there was no RAC × biological type interaction which led authors to conclude that RAC affected growth performance and carcass characteristics in a similar manner across these three distinct biological types (Gruber et al., 2007).

While biological type may not affect the response of growth performance and carcass characteristics to RAC supplementation, implant strategy may play a role in the response. In a series of experiments, aggressive and conservative implant strategies were followed when RAC was supplemented to heifers. When the conservative protocol was administered, RAC supplementation increased ADG, G:F ratio, HCW, and ribeye area. In addition, RAC decreased 12^{th} rib fat and yield grades. When the aggressive protocol was combined with RAC supplementation, RAC improved G:F ratio but did not affect other growth performance or carcass characteristics. The authors hypothesized that steroid implants could affect the sensitivity of BAA receptors and interfere with the response to RAC. Therefore, they concluded

that implant strategy in addition to other management strategies must be considered to maximize the response to RAC (Sissom et al., 2007).

The effects of RAC supplementation level (0, 100, 200 mg•hd^{-1}•d^{-1}) and duration of supplementation (28, 35, 42 days) on growth performance and carcass characteristics were evaluated next. Data found that there was no RAC dose × duration interaction. However, as RAC dose was increased, final body weight, ADG, and G:F ratio increased. Ractopamine duration tended to cause quadratic increases in dry matter intake, G:F ratio, and final body weight. There was a significant quadratic effect for ADG and supplementation duration with increases detected up to day 35, but no further increases thereafter. When the two RAC dosage levels were separated and analyzed independently of one another, data indicated that the 100 mg•hd^{-1}•d^{-1} dosage group had linear increases in dry matter intake, ADG, and G:F ratio. However, the 200 mg•hd^{-1}•d^{-1} supplementation group had quadratic increase in the same parameters. The authors concluded that the maximum benefit for growth performance was reached at day 42 for the 100 mg•hd^{-1}•d^{-1} supplementation group and day 35 for the 200 mg•hd^{-1}•d^{-1} supplementation group. The authors hypothesized that the differences between the two groups was due to receptor desensitization experienced when feeding high levels of BAAs. Ractopamine had only a modest effect on carcass characteristics. In agreement with the growth performance data, there were no RAC dose × duration interactions for carcass characteristics. Hot carcass weight increased linearly as RAC dose was increased, but tended to act quadratically as duration of supplementation increased. A tendency for linear increases in ribeye area due to increases in dosage was the only other carcass characteristic affected by RAC. The authors concluded that RAC improves growth performance and carcass weight, and should be fed for a shorter period if fed at a high dose (Abney et al., 2007).

Data from Schroder et al. (2004a,b) and Sissom et al. (2007) reported that heifers do not respond to RAC in a similar manner to steers. Therefore, two experiments were conducted examining the response of heifers to RAC. In the first experiment, RAC was fed for 28 days prior to harvest at the manufacturer's recommended dose of 200 mg•hd^{-1}•d^{-1}. Feeding RAC to heifers in this manner did not affect growth performance except for a tendency to increase G:F ratio. All carcass characteristics and meat quality characteristics, including Warner-Bratzler shear force, purge loss, cook loss, and L*a*b* colorimetric values during retail display were unaffected by RAC supplementation. Due to the lack of response by the heifers when RAC was fed according to the manufacturer's recommendation, three alternate feeding regimens were tested. These regimens included feeding 300 mg•hd^{-1}•d^{-1} for the last 28 days, feeding 200 mg•hd^{-1}•d^{-1} for the last 42 days, and a step-up method that involved increasing the dosage rate 100 mg•hd^{-1}•d^{-1} every 14 days for the last 42 days. Feeding RAC using these protocols had minimal effects on growth performance. The 300 mg•hd^{-1}•d^{-1} dosage level reduced dry matter intake. Ractopamine supplementation improved G:F ratio and ADG, but all RAC feeding regimens were not different. While differences between the feeding regimens did not occur for growth performance, there were numerical differences between the 28 day and 42 day feeding periods. The authors also noted that feeding the step-up method appeared to be more effective than feeding constant doses for 28 days. However, this method did not cause greater improvements over feeding 200 mg•hd^{-1}•d^{-1} for 42 days. Therefore, the authors suggested that feeding RAC for a longer period, not a higher dosage, may be needed to elicit a greater response. In agreement with the first experiment, all carcass characteristics were unaffected by RAC supplementation (Quinn et al., 2008).

The most recent study published involving the supplementation of RAC to beef cattle examined the effects of management system on the RAC response. In two experiments, conventionally raised cattle and naturally raised cattle were compared in a 2 × 2 factorial arrangement. Conventionally raised cattle were administered a Revalor-S implant and fed a monensin/tylosin feed additive. Naturally raised cattle were neither implanted nor fed the feed additive. In experiment one, RAC was supplemented for 37 days and increased ADG. Gain to feed ratio was greater in conventionally raised steers fed RAC than conventionally raised steers not fed RAC. Gain to feed ratio also was greater in naturally raised cattle fed RAC than naturally raised cattle not fed RAC. For carcass characteristics, RAC lowered yield grade and quality score and tended to increase ribeye area. These findings led the authors to conclude that a synergistic effect may occur between the growth promotants employed. In addition, steers implanted before RAC supplementation had a greater ADG and G:F ratio, which indicated that management practices utilized before RAC administration affected the RAC response.

In the second experiment, a management system × RAC interaction was detected for ADG, G:F ratio, and HCW. During that last 28 days of feeding, the conventionally raised cattle supplemented RAC had a greater ADG and G:F ratio, and heavier carcasses than the naturally raised cattle not fed RAC. The authors concluded that that the management system and RAC worked synergistically to regulate growth and the management system followed in time period before RAC supplementation caused the observed differences. The authors hypothesized that this was caused by the ability of the implants in the conventional treatments increased satellite cell proliferation and enhanced muscle fiber hypertrophy (Winterholler et al., 2008).

Effect of β-Adrenergic Agonists on Fresh Meat Shelf-Life

Consumers visually evaluate numerous factors when considering which product to purchase in the retail display case (MacKinney et al., 1966). These factors may include portion

size, leanness, ease of preparation, and color. Hedrick et al. (1994) and Kropf (1980) identified color as the single most important visual component that determines if a consumer will purchase a meat product. Because BAAs, including RAC, shift muscle fiber isoforms, supplementation of RAC may affect color and shelf-life. Currently, little data exists documenting the effects of RAC on fresh meat color and shelf-life.

Ractopamine supplemented to pigs has little to no effect on the color or shelf-life of pork products. Watkins et al. (1990) reported mixed results when RAC was fed to finishing pigs at dosage levels of 2.5, 5, 10, 20, 30 ppm. In their initial experiment, RAC did not affect the color of the loin. However, in their second experiment, RAC fed at 10 and 15 ppm caused loins from these pigs to become darker. In agreement with the Watkins et al. (1990) first experiment, Stites et al. (1991) found that the RAC had no effect on loin color when fed at 5, 10, and 15 ppm. Stites et al. (1994) also examined these levels of supplementation and their effect on cured ham color. The researchers found that RAC did not affect cured color, cured color uniformity, or the amount of discoloration. In the same study, RAC did not affect loin chop lean color, surface discoloration, or overall appearance during a four day retail display study.

Recently, Apple et al. (2008) studied the effect of RAC supplementation on the shelf-life properties of loin chops. Pigs were fed 10 mg/kg of RAC during the final 35 days of feeding and loin chops were displayed under retail conditions for five days. Across the five days of display, RAC chops received higher subjective color scores, which indicates that RAC caused the chops to become darker. This finding was in disagreement with Armstrong et al. (2004) and Carr et al. (2005), who each found that RAC did not affect either Japanese of American pork color scores. In addition, RAC caused chops to become darker, less red, less yellow, and less vivid in color than controls. Others have also found that RAC supplemented between 5 and 20 mg/kg

decreases redness and yellowness in loin chops (Armstrong et al., 2004; Carr et al., 2005). These findings led the authors to conclude that over a five day retail study, RAC fed at 10 mg/kg can enhance loin chop muscle quality.

Color and shelf-life data from studies with RAC and the other beef cattle approved BAA, zilpaterol, differ from study to study. Dietzel (1990) reported that supplementing RAC at 30 ppm during the last 45 days of feeding produced steaks with a subjectively brighter cherry red lean color after four days of display when compared to untreated steaks. Additionally, steaks from RAC fed steers decreased in overall appearance slower than steaks from non-treated steers. In agreement with this study, Avendano-Reyes et al. (2006) compared steaks from ractopamine (300 $mg \bullet hd^{-1} \bullet d^{-1}$) and zilpaterol (60 $mg \bullet hd^{-1} \bullet d^{-1}$) supplemented animals to control steaks and found that control steaks became darker than steaks from BAA supplemented animals on day 5 of a retail display period. However, Quinn et al. (2008) supplemented heifers with 200 $mg \bullet hd^{-1} \bullet d^{-1}$ of RAC for 28 days prior to harvest and found that the five day retail L*a*b* values of longissimus muscle steaks were unaffected. Therefore, this could indicate that larger dose or longer supplementation time may be needed to affect beef steak color and shelf-life.

Other studies conducted with the BAA zilpaterol-HCl have contradictory findings concerning its effect on color and shelf-life. Brooks et al. (2008) summarized a series of studies concluding that this agonist has no detrimental effect on color scores and can improve color stability and shelf-life. In their summary, Brooks et al. (2008) reported that several studies found that zilpaterol-HCl improved carcass color scores and resulted in muscles that were more cherry-red in color. Other studies cited indicate that zilpaterol-HCl improved shelf-life and reduced the levels of metmyoglobin accumulation on the surface of steaks. Additional research found that zilpaterol-HCl supplementation produced steaks with a more desirable lean color. In contrast to

this report, Neill et al. (2008) found that steaks originating from the knuckle of implanted cull cows fed zilpaterol-HCl were darker and had more discoloration on day 5 of display than cows that were grass fed, concentrate fed, and concentrate fed with the inclusion of zilpaterol. This could suggest that zilpaterol-HCl has a differential effect on the color and shelf-life of various muscles. While certain hypothesis and conclusions can be drawn from the data already published, more research is needed to fully understand the effects of BAAs on the color and shelf-life of different muscles of the beef animal.

CHAPTER 3
EFFECT OF RACTOPAMINE-HCl AND TRENBOLONE ACETATE ON LONGISSIMUS MUSCLE FIBER AREA, DIAMETER, AND SATELLITE CELL NUMBERS IN CULL BEEF COWS

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Introduction

Ractopamine HCl (**RAC**), a beta-agonist with established nutrient partitioning capabilities, promotes skeletal muscle accretion at the expense of fat deposition (for review see Mersmann, 1998). Many studies exist detailing the positive effects of RAC on swine performance and carcass composition (Dunshea et al., 1993, 1998; Sainz et al., 1993). At the cellular level in pigs, beta-agonists increase the size of type IIB muscle fibers. Cross-sectional area and fiber diameters were larger in pigs fed RAC (Aalhus et al., 1992) and an increase in the relative amount of myosin IIB was apparent (Depreux et al., 2002). A second effective means of altering feed efficiency and carcass composition is the use of steroid implants. In growing steers, treatment with Revalor-S (trenbolone acetate + estradiol; **TBA**) resulted in an increase in type IIB fibers without a change in the size or number of type I muscle cells (Fritsche et al., 2000).

Satellite cells, or muscle stem cells, are responsible for postnatal muscle fiber growth and repair (Mauro, 1961; Schultz et al., 1978). This typically quiescent population of cells lies adjacent to the muscle fiber under the basal lamina and is identified *in vivo* by their expression of Pax7. Pax7 is a member of the paired-box family of transcriptional mediators and is implicated in the establishment of the satellite cell lineage (Seale et al., 2000). Mice devoid of *Pax7* exhibit severe muscle size and functional deficits that are due to an absence of satellite cells (Mansouri et al., 1996, Seale et al., 2000). The animals die within 3 to 4 wk of age. Pax7 does not alter

proliferation rates but does inhibit satellite cell differentiation and apoptosis (Olguin and Olwin, 2004; Relaix et al., 2006; Zammitt et al., 2006).

Due to limited information regarding aged bovine muscle fiber size, satellite cell numbers and growth capabilities, we examined LM fiber morphometrics and myonuclei numbers in cull cows fed RAC and TBA.

Materials and Methods

Animals and Diets

This experiment was approved by the University of Florida Institutional Animal Care and Use Committee. Ninety-two crossbred beef cows (11 yr ± 1.8) culled from a commercial cow-calf operation in south Florida (Lykes Bros., Okeechobee, FL) were shipped by two different truck loads to a feeding facility near Gainesville, FL on the same day. Upon arrival, the cattle were weighed and the general health was evaluated. Cows were given individual ID tags, dewormed with a generic anthelmintic (Agri Laboratories, Ltd., St. Joseph, MO), and tail switches were trimmed. Cows were blocked by BW on arrival into two replicates (heavy and light) and randomly assigned to treatments according to a 2 x 2 factorial arrangement. At the beginning of the study light cows weighed 370 kg and heavy cows weighed 418 kg.

Cows were fed in four pens with implant status and dietary treatment as the main effects. All diets were fed ad libitum in self feeders. The BCS of all cows on arrival was uniform (4.3 ± 0.03; Carter et al., 2006). One half of the cows in each pen were implanted with Revalor-IS (80 mg trenbolone acetate plus 16 mg estradiol; Intervet, Millsboro, DE), while the remainder received no implant. The basal diet was fed to one half of the cows (two pens) for the duration of the 92 d on feed. The remaining one half (two pens) were fed the same basal diet from d 0 to 55. On d 56, a pelleted supplement containing RAC was added to the basal diet, delivered to empty self-feeders, and fed ad libitum for the remaining 35 d on feed. The basal diet consisted

of (DM basis) soybean hulls (21.1%), citrus pulp (19.7%), cracked corn (14.4%), wheat middlings (14.2%), cottonseed hulls (12.7%), cottonseed meal (7.0%), liquid molasses (7.0%), vitamins and minerals (included sodium bicarbonate) (2.1%), tallow (1.3%), and urea (0.4%). The diet provided 87.6% DM, 14% crude protein (DM basis) and 79.5% TDN and was formulated to meet the nutrient requirements of a non-pregnant, non-lactating, beef cow predicted to gain 2.06 kg/d. The pelleted, premixed supplement (Type B premix) consisted of wheat middlings (97.6%) and ractopamine HCl (2.4%) (Optaflexx 45; Elanco Animal Health, Greenfield, IN) and was formulated to provide approximately 15 ppm when combined at the proper rate in our basal diet depending on DMI (projected to be approximately 13.6 $kg \bullet hd^{-1} \bullet d^{-1}$). This study was designed to mimic feedyard standard housing and management practices. Individual animal intake was not monitored and it is acknowledged that the implanted cows may have had a greater intake. Thus, the projected range of RAC was between 15 ppm and 16.5 ppm. The type B premix with RAC was randomly sampled and analyzed for the concentration of the experimental compound before blending and feeding to ensure accurate delivery of the formula at the prescribed rate. Analytical results indicated that the B-premix contained on average 2.15 g/kg of RAC (As-fed basis) and would adequately provide the target level of RAC.

The basal diet also included an ionophore [Rumensin 80 (monensin granulated); Elanco Animal Health, Greenfield, IN] formulated at the rate of 22 mg/kg of feed. Feed samples were collected randomly over the feeding period and analyzed for monensin concentration, which averaged 22.22 mg/kg.

Harvesting and Sample Collection

On d 92, cows were harvested in a commercial slaughter facility located in Center Hill, FL under USDA inspection. Preharvest BW were 502 kg for light cows and 522 kg for the heavy replicate. Following a 48 h chill period and carcass data collection, 10 wholesale ribs were

randomly selected from each treatment group (n = 40). The sixth rib steak from each wholesale rib section was removed. Two 1 cm x 1 cm x 1 cm portions of the sixth rib LD were suspended in OCT tissue freezing medium (Fisher Scientific, Hampton, NH), frozen by submersion in super-cooled isopentane, and stored at -80°C.

Immunohistochemistry

Three serial cryosections (12 μm), one for each fiber isoform, were collected on frost resistant slides (Fisher Scientific, Hampton, NH) for each LD sample. Two sets of serial cryosections were collected for each animal and the protocol of Watson et al. (2003) was followed with modifications. Non-specific antigen sites were blocked in 5% horse serum in PBS for 20 min at room temperature. Cryosections were incubated for 60 min at room temperature in primary antibodies. Antibodies and dilutions were: α-dystrophin (Abcam, Cambridge, MA) 1:50; undiluted supernatant myosin heavy chain type 1 (BAD.5, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA); myosin heavy chain type 2A (SC.71, Developmental Studies Hybridoma Bank); myosin heavy chain type 2B (BF.F3, Developmental Studies Hybridoma Bank); Pax7 (Developmental Studies Hybridoma Bank) 1:5 cultured supernatant. The myosin heavy chain antibodies were directed toward bovine skeletal muscle myosin (Schiaffino et al., 1989).

After extensive washing with PBS, tissues were incubated for 40 minutes with goat anti-mouse Alexa Flour 568 (1:500; Invitrogen, San Diego, CA) for α-dystrophin or goat anti-mouse biotin (1:100; Vector Laboratories, Burlingame, CA) followed by steptavidin Alexa Flour 488 (1:500; Invitrogen) for Pax7 and myosin heavy chain isoform detection. Following Pax7 immunostaining, Hoechst 33245 (1 μg/ml in PBS) was used to identify total nuclei (Example

microphotograph in Appendix A). Fiber associated nuclei (**FAN**) were visualized with propidium iodide (1 µg/ml; Invitrogen; Example microphotograph in Appendix B).

After a final PBS wash, slides were cover slipped and immunostaining was evaluated using a Eclipse TE 2000-U stage microscope (Nikon, Lewisville, TX) equipped with an X-Cite 120 epifluorescence illumination system (EXFO, Mississauga, Ontario, Canada). Images were captured at 100X magnification using a DXM 1200F digital camera (Nikon) and analyzed for individual muscle fiber area and diameter and total number of fiber associated nuclei per field using the NIS-Elements computer system (Nikon). For each set of serial cryosections, 4 images from the same area of each cryosection was collected for each myosin heavy chain isoform (Figure 3-1). Fibers that were reactive with the antibody to the specific myosin heavy chain isoform were counted, and fiber area was defined as the region constrained by α-dystrophin immunostaining. Diameter was measured by the computer system rotating every 90 degrees around the fiber, taking a diameter measurement, and averaging the measurements. For each animal, a minimum of 475 fibers were measured and used for analysis. Fiber associated nuclei was defined as propidium iodide stained cells contained within a α-dystrophin boundary. Nuclei that were identified with Hoechst dye labeled as Pax7 positive were counted as being a satellite cell.

To ensure collecting samples 48 h postmortem did not have an effect on satellite cell detection, a validation study was conducted. Satellite cell numbers were measured in 3 LD immediately after slaughter and at 24, 48 and 72 h of chill. No differences in Pax7 immunopositive cells were observed (Figure 3-2).

Statistics

The study was designed as a randomized complete block design with individual carcasses of the 4 different feeding regimens as the experimental unit (Matulis et al., 1987; Cranwell et al.,

1996b; Schnell et al., 1997). Fiber frequencies were tabulated and compared by chi-square analysis using PROC FREQ of SAS (SAS Inst. Inc., Cary, NC, 2002). Treatment group frequencies within a fiber type were compared to one another by a two sample t-test for proportions. Data for fiber area and diameter were sorted and analyzed by individual fiber type, while fiber-associated nuclei and Pax7 nuclei were not sorted. Data were analyzed with the PROC MIXED procedure of SAS where implant status, dietary treatment and their interaction were the fixed effects. Random effects included BW replicate, truck load, and animal within treatment. Each combination of BW replicate and truck load were grouped and used in the random statement. Pair-wise comparisons between the least square means of the factor levels were computed by using the PDIFF option of the LSMEANS statement. The PROC UNIVARIATE procedure of SAS was used to generate histograms and analyze distributions of fiber diameter and area within each treatment group for each fiber type.

Results

Muscle fiber types were measured using antibodies specific to myosin heavy chain type I, IIA and IIB isoforms. No type IIB immunoreactivity was observed suggesting that LM was comprised solely of type I and IIA fibers (Figure 3-1). Muscle fiber diameters were measured following co-localization of myosin and dystrophin. There was a significant ($P < 0.01$) RAC x TBA interaction for type I fibers. The main effects of TBA and RAC increased ($P < 0.05$) the diameter and computed cross-sectional area (**CSA**) of type I fibers (Table 3-1). Feeding RAC to TBA implanted cull cows did not further increase ($P > 0.05$) the LM fiber diameter or CSA. No change ($P > 0.05$) in the CSA or diameters of type II fibers occurred in response to TBA, RAC or TBA + RAC. No differences ($P > 0.05$) in the percentage of type I and type II fibers were observed across the CON, RAC, and TBA treatments (Table 3-1). There was an increase ($P < 0.001$) in the percentage of type II fibers in the LM of cull cows treated with RAC/TBA.

Size-frequency histograms of CSA were generated for type I and type II LM fibers. Trenbolone acetate and TBA + RAC caused a shift in numbers of type I fibers with larger CSA than CON (Figure 3-3). Curve of best fit for type II muscle fiber histograms do not differ (P = 0.18) between the groups (Figure 3-4).

The numbers of nuclei contained within the dystrophin boundary were measured as an index of hypertrophy. Fiber associated nuclei were not affected ($P > 0.05$) by any of the treatments given (Table 3-2). Satellite cell number, as measured by anti-Pax7 at 48 h post-mortem, did not change ($P > 0.05$) in response to any treatment (Table 3-2).

Discussion

Growing pigs fed RAC demonstrate an increase in loin eye area that is a result of increased type II fiber size. Coincident with the larger fiber diameter and CSA is a shift from the fast oxidative (type IIA) to the fast glycolytic (type IIB) metabolic phenotype (Aalhus et al., 1992). A two-fold increase in the numbers of type IIB fibers, at the expense of the type IIA fibers, was recorded in RAC supplemented hogs (Depreux et al., 2002). In the present study, neither type IIB nor type IIX muscle fibers were detected by immunocytochemistry. The population of type IIA muscle fibers remained constant at approximately 68% of the total fibers in cattle fed RAC or implanted with TBA. A greater percentage of type IIA fibers at the expense of type I fibers was observed in TBA implanted cows fed RAC. This result is intriguing in light of our inability to detect a change in the proportion of type II fibers in cows fed RAC only. In cattle and mice, it is well established that beta agonists increase the percentage of type IIA fibers at the expense of type I fibers (Vestergaard et al., 1994; Rajab et al., 2000; Bricout et al., 2004). By contrast, anabolic agents have no effect on the distribution of type I or type IIA fibers (Ono et al., 1996; Fritsche et al., 2000). The underlying mechanism of action of RAC/TBA that elicits a change in myosin isoforms remains unknown and warrants further investigation.

The type IIA antibody used in this study reportedly cross reacts with myosin IIX and IIB in cattle (Duris et al., 2000) and type IIX fibers in swine (Depreux et al., 2000). Based on the assumption of that all type II fibers are immunoreactive, 60% of the LM fibers in cull cows are classified as fast, a value in agreement with prior publications. Using enzymatic techniques, Brandstetter et al. (1998) identified the percentage of type I, type IIA and type IIB fibers as 25%, 25% and 50%, respectively, in the LM. Fritsche et al. (2000) reported a similar percentage with 20 to 30% of fibers classified as oxidative and approximately 55% of the total fibers present as fast glycolytic fibers. In a similar manner, the inability to detect type IIB fibers may be a limitation of the immunodetection method. Watson et al. (2003) failed to detect type IIB fibers in harbor seals using the same antibody. Duris et al. (2000) reported this antibody demonstrates a low specificity for type II myosin heavy chain in bovine tissues and indicated that anti-myosin IIB (N3.36) is a suitable alternative. However, monoclonal N3.36 failed to detect type IIB fibers in the LM of cull cows fed RAC. Our inability to detect myosin type IIB fibers agrees with Tanabe et al. (1998) and Toniolo et al. (2005), who were unable to establish the presence of myosin IIB in bovine LM by semi-quantitative RT-PCR. Chikuni et al. (2004) also reported a lack of myosin IIB mRNA as measured by real-time PCR, and hypothesized the absence of this isoform may explain the differences between beef and pork. Thus, the inability of RAC to shift myosin expression to the fastest isoform may be unique to cattle. Alternatively, fiber type shifts in response to RAC may occur only in muscle that normally expresses limited amounts of myosin IIB.

Quartile analysis of all type II fibers demonstrates no apparent difference in the percentage of fibers present with a larger diameter in RAC cull cows by comparison with CON suggesting that the response of IIA fibers was minimal. Indeed, CON animals appear to have the greatest

percentage of large diameter fibers that may represent type IIB. The unresponsive nature of type II fibers of cull cows to RAC in the present study may reflect the muscle environment. Finishing hogs fed normal or supraphysiological levels of RAC contained an equivalent amount of myosin IIA in the LM as control animals. By contrast, the semitendinosus muscle contained less myosin IIA in response to RAC (Depreux et al., 2002). Alternatively, the resident type II population may be refractile to growth enhancing agents due to the advanced age of the animal. The differential response of bovine and porcine type II muscle fibers to the beta agonist is intriguing and warrants further investigation.

A substantial increase in the size of type I fibers was evident in cows treated with RAC or TBA. Administration of TBA to cull cows increased LM area and carcass fat-free lean (Cranwell et al., 1996a). In a similar manner, implantation of feedlot steers with TBA increased type I and IIA CSA in the LM (Hughes et al., 1998). Thus, the larger diameter type I fibers in cull cows receiving TBA reflects previous reports. Conversely, an increase in LM type I CSA by RAC supplementation is novel. No change in the size of type I fibers is apparent in finishing hogs supplemented with RAC (Aalhus et al., 1992). Longissimus muscle type I fibers from lambs fed cimaterol showed no change to a modest 15 percent increase in fiber CSA (Beermann et al., 1987; Kim et al., 1987). The thirty percent increase in type I CSA found in RAC supplemented cows suggests that this population of cells are two to three times more responsive in cattle than other species. Further support for specie differences is reflected by a 35 percent larger type I CSA in bulls fed cimaterol (Vestergaard et al., 1994). However, cull cows are unique in that their type II fiber population is completely refractile to RAC induced hypertrophy whereas cimaterol stimulated hypertrophy in bulls (Vestergaard et al., 1994). The mechanism behind the differential response in cattle may be a reflection of the age of the animal. During

aging in humans, the numbers of type IIA/X muscle fibers and size are reduced (Lee et al., 2006; Verdijk et al., 2007). Type I fiber CSA remains unchanged largely but the percentage of type I fibers are increased. In addition, the ability of the muscle to respond to hypertrophic events is altered in extreme age (for review see Carmeli et al., 2002). Advanced age in rodents, birds and humans demonstrate an impaired ability to increase in size that is associated with reduced type II muscle fiber numbers (Carson et al., 1995; Blough and Linderman, 2000; Short et al., 2005; Lee et al, 2006). The percentage of type I and II fibers is established by 24 mo of age in cattle, irrespective of breed, and the LM is comprised predominantly of type I and IIB (Wegner et al., 2000; Kirchofer, et al., 2002). In the event that type II fibers are limited in their protein synthetic capacity and are intolerant to hypertrophic stimuli, the nutrients supplied by diet coupled with the partitioning agents leads to an excess of available substrate for type I fiber growth.

One of the primary reasons for supplementing livestock with RAC or TBA is to improve carcass value. Schroeder et al (2005b,f) reported an increase in ribeye area and fat-free lean in steers and heifers fed RAC. Therefore, based on the findings above we predicted that cull cows receiving 15 ppm RAC daily for 35 d would possess a larger REA. However, no improvement in carcass characteristics, including REA, due to RAC or TBA supplementation were detected (Carter et al., 2006). This may be a reflection of an unresponsive type II fiber population. Approximately 30% of the total number of fibers was present as type I. Based on the 30% increase in type I size found in young bulls (Vestergaard et al., 1994), we would predict a minimal 9% increase in REA. This small change may require more animals to reach statistical significance.

Postnatal skeletal muscle growth is accomplished through the satellite cell population. These normally quiescent muscle stem cells become mitotically active, proliferate and fuse into

existing muscle fibers (for review see Collins, 2006). The number of satellite cells declines with age and the activation potential of these cells are reduced in older individuals (Collins and Partridge, 2005). In aged rats and elderly humans, satellite cells represent one to two percent of the total myonuclei (Gallegly et al., 2004; Sajko et al., 2004; Brack et al., 2005). The number of Pax7 immunopositive satellite cells in cull cows represents approximately 1% of the total number of myonuclei, in close agreement with rodent data. Satellite cells isolated from TBA implanted steers exit the dormant state sooner than their contemporaries suggesting that the anabolic steroid affects self-renewal and subsequent proliferation. In addition, these cells fused into larger muscle fibers in vitro (Johnson et al., 1998). The enhanced myogenic capabilities may account for the larger fiber sizes found in TBA implanted cull cows. Alternatively, TBA increased circulating levels of IGF-I and autocrine synthesis of the growth factor (Thompson et al., 1989; White et al., 2003; Kamanga-Sollo, et al., 2004). It is possible that the increased type I fiber diameters and calculated CSA are a product of elevated IGF-1 activities.

Satellite cells proliferate, differentiate and fuse with the muscle fibers to provide FAN for increased contractile gene expression and maintenance of the myonuclear domain (Aberle et al., 2001). The lack of an increase of FAN found in all the supplemented cows indicates that the two growth promotants may act through a similar pathway of altered protein synthesis and/or degradation rates. It was reported that RAC stimulates protein synthesis without an apparent effect on satellite cell cycle kinetics or fusion (Shappell et al., 2000). Beta-agonists may also augment nutrient supply to the muscle cell by increasing blood flow. Muscle accretion is supported and possibly bolstered by the enhanced delivery of substrates and energy needed for protein synthesis (Mersmann, 1998). In several swine and cattle studies, the mechanism of muscle growth due to ractopamine supplementation was attributed to enhanced protein synthesis

(Smith et al., 1987; Dunshea et al., 1993, 1998; Williams et al., 1994). Beermann et al. (1987) and Kim et al. (1987) reported that DNA concentration per gram of protein was less in beta-agonist supplemented lambs and both groups concluded that muscle growth was due to a reduction in protein degradation and independent of satellite cell activity. A similar observation was reported for rats (Maltin et al., 1986) and lambs (Bohorov et al., 1987) fed clenbuterol. The discrepancies between increased protein synthesis or reduced degradation rates may be a reflection of the beta agonist fed and the beta-receptor isoform activated.

Conclusion

Supplementing cull cows with either RAC or TBA alone, or the combination increased LM fiber CSA and diameter for type I fibers, while having no effect on type II fibers. There were no fiber type shifts between the different myosin heavy chain isoforms due to the presence of TBA or RAC. Fiber associated or satellite cell numbers were not affected by the RAC or TBA treatments. The lack of affect on FAN and constant satellite cell numbers suggests that any hypertrophy occurred due to changes in protein synthesis and/or degradation rates. It is hypothesized that because protein synthesis is limited in older animals, this prevented the response to both TBA and RAC normally seen in younger animals.

Table 3-1. Longissimus muscle type I and type II fiber percentage and least square means of fiber cross-sectional area and diameter from cull cows fed four different feeding regimens

Treatment	Type I			Type II		
	Percentage[1]	Area (μm^2)	Diameter (μm)	Percentage[1]	Area (μm^2)	Diameter (μm)
CON[2]	32.35^a	2432.97 ± 73.56^a	54.61 ± 0.73^a	67.65^a	5087.12 ± 261.30	79.70 ± 1.56
RAC[3]	31.54^a	3191.89 ± 99.18^b	62.62 ± 0.98^b	68.46^a	3626.91 ± 253.48	66.55 ± 1.52
TBA[4]	32.35^a	3678.72 ± 190.40^c	66.89 ±1.78^c	67.65^a	4690.68 ± 446.62	75.23 ± 3.60
RAC/TBA[5]	28.78^b	3615.42 ± 96.36^c	66.58 ± 0.93^c	71.22^b	4830.49 ± 241.12	76.13 ± 1.50

Means within a column with a different letter are significantly different ($P < 0.05$).
[1]Percent fiber type frequency detected by monoclonal antibody immunohistochemistry.
[2] Cull cows fed the control diet (n = 10).
[3]Cull cows fed the control diet plus Optaflexx (15 ppm ractopamine-HCl) supplement during the last 35 days on feed (n = 10).
[4]Cull cows implanted with Revalor-IS (80 mg trenbolone acetate plus 16 mg estradiol) and fed the control diet(n = 10).
[5]Cull cows implanted with Revalor-IS, fed the control diet plus Optaflexx supplement during the last 35 days on feed (n = 10).

Table 3-2. Least squares means of LM fiber associated nuclei per fiber[1], and satellite cells per hundred fibers[2] of cull cows fed four different feeding regimens

Treatment	Fiber Associated Nuclei	Satellite Cells
CON[3]	4.69 ± 0.75	4.63 ± 0.91
RAC[4]	4.62 ± 0.77	3.20 ± 0.88
TBA[5]	5.16 ± 0.71	5.89 ± 0.70
RAC/TBA[6]	4.97 ± 0.76	4.24 ± 0.64

[1]Total nuclei counted in a field divided by number of fibers counted per field (field equals 381 mm^2).
[2] Total satellite cells counted in a field divided by number of fibers counted per field multiplied by 100(field equals 381 mm^2).
[3]Cull cows fed the control diet (n = 10).
[4]Cull cows fed the control diet plus Optaflexx (15 ppm ractopamine-HCl) supplement during the last 35 days on feed (n = 10).
[5]Cull cows implanted with Revalor-IS (80 mg trenbolone acetate plus 16 mg estradiol) and fed the control diet(n = 10).
[6]Cull cows implanted with Revalor-IS, fed the control diet plus Optaflexx supplement during the last 35 days on feed (n = 10).

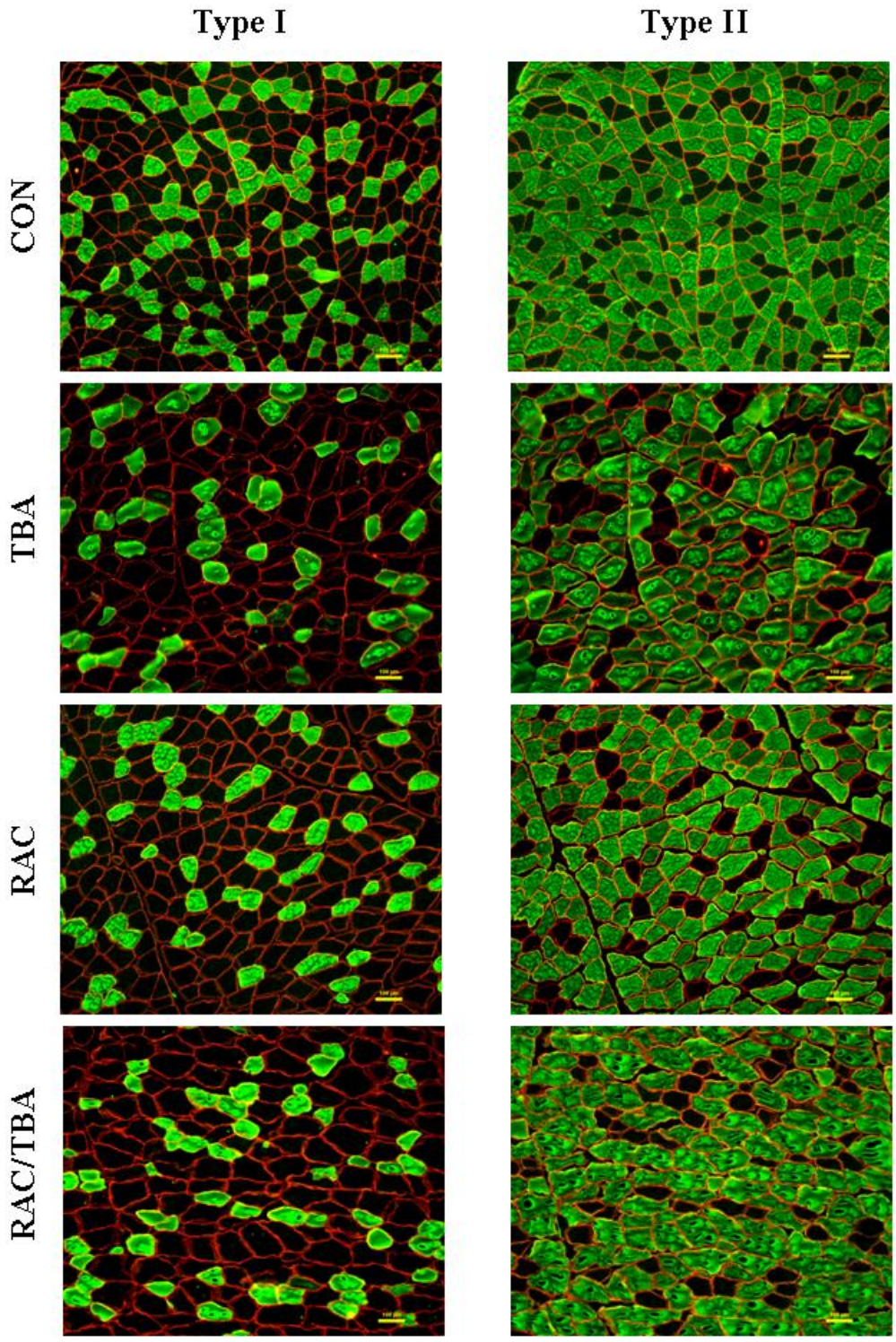

Figure 3-1. Representative photomicrographs of LM fibers immunostained for type I and type II fibers from (CON) control diet fed cows, (RAC) control diet + RAC fed cows, (TBA) control diet + implant fed cows, and (RAC/TBA) control diet + RAC + implant fed cows. Scale bar equals 100 μm.

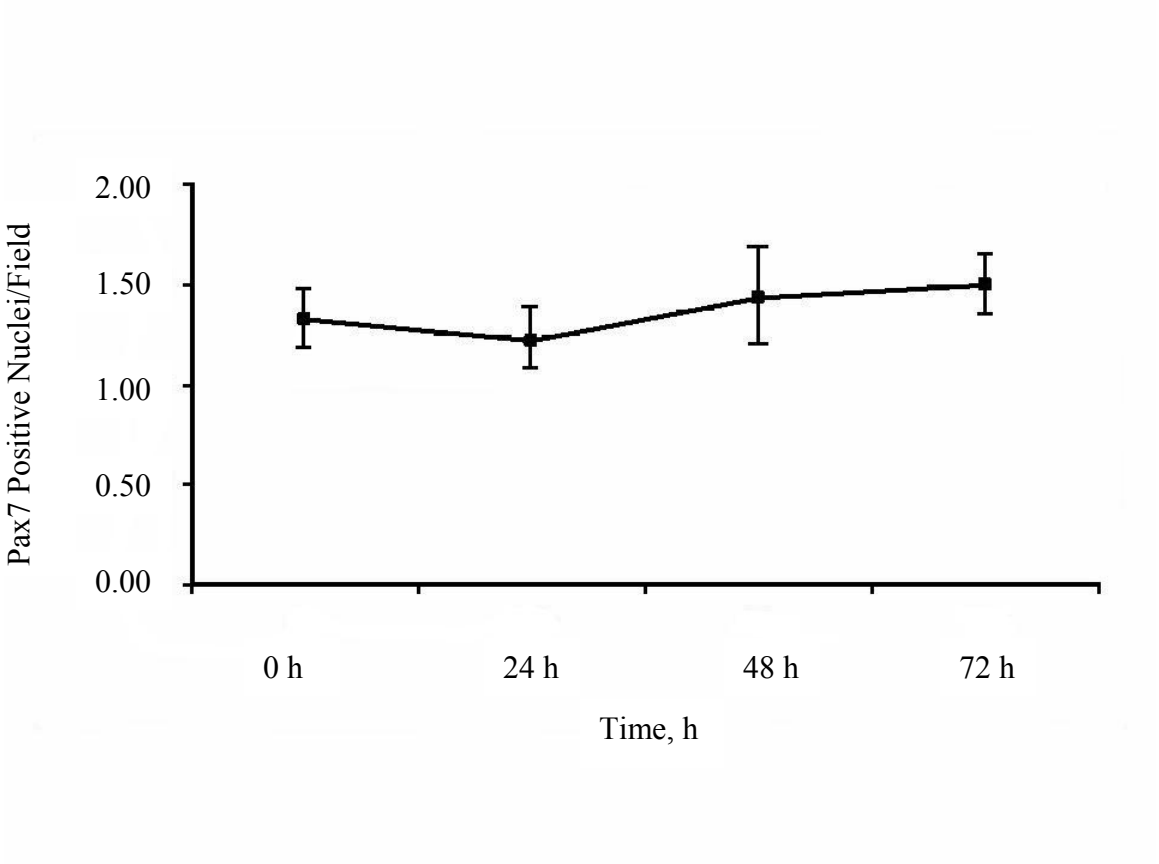

Figure 3-2. Number of Pax7 positive nuclei counted per field during a 72 h period postmortem. Samples were taken at 0, 24, 48, and 72 hours postmortem and subjected to the Pax7 staining protocol used in the present study. Area of field equals 41.5 mm^2.

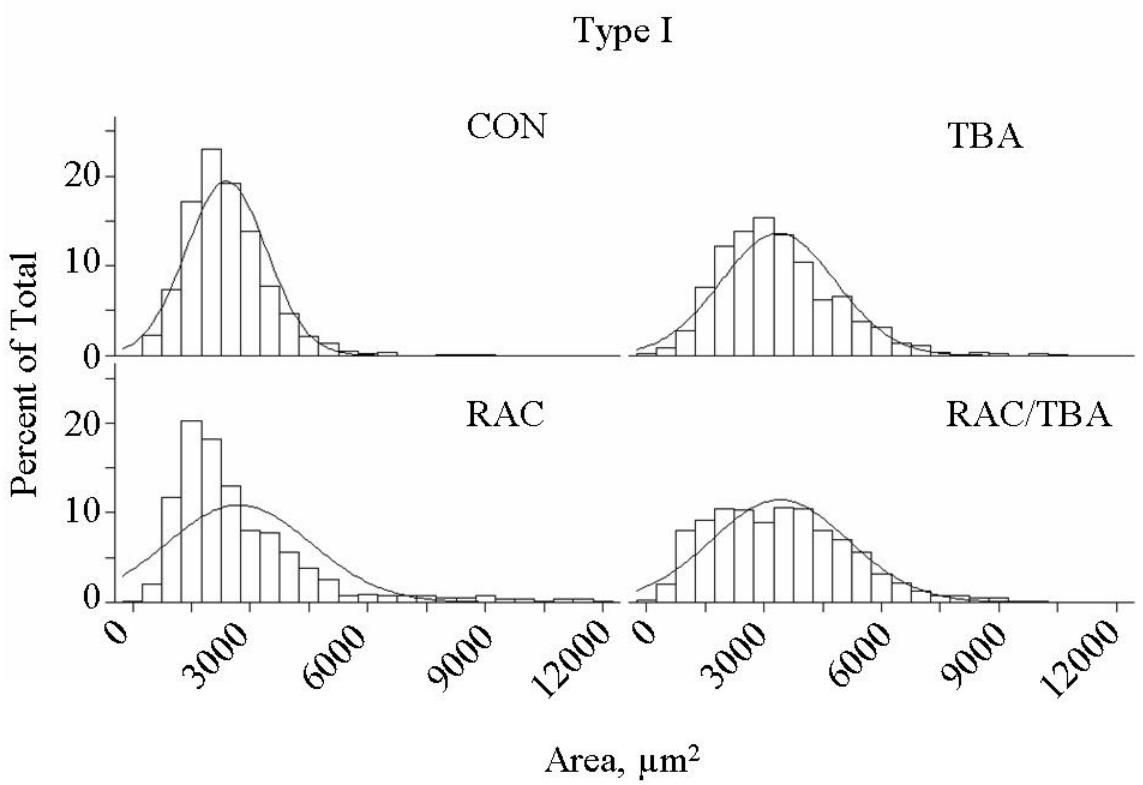

Figure 3-3. Histograms of LM fiber cross-sectional areas of all type I fibers sampled from (Control) control diet fed cows, (RAC) control diet + RAC fed cows, (TBA) control diet + implant fed cows, and (RAC/TBA) control diet + RAC + implant fed cows.

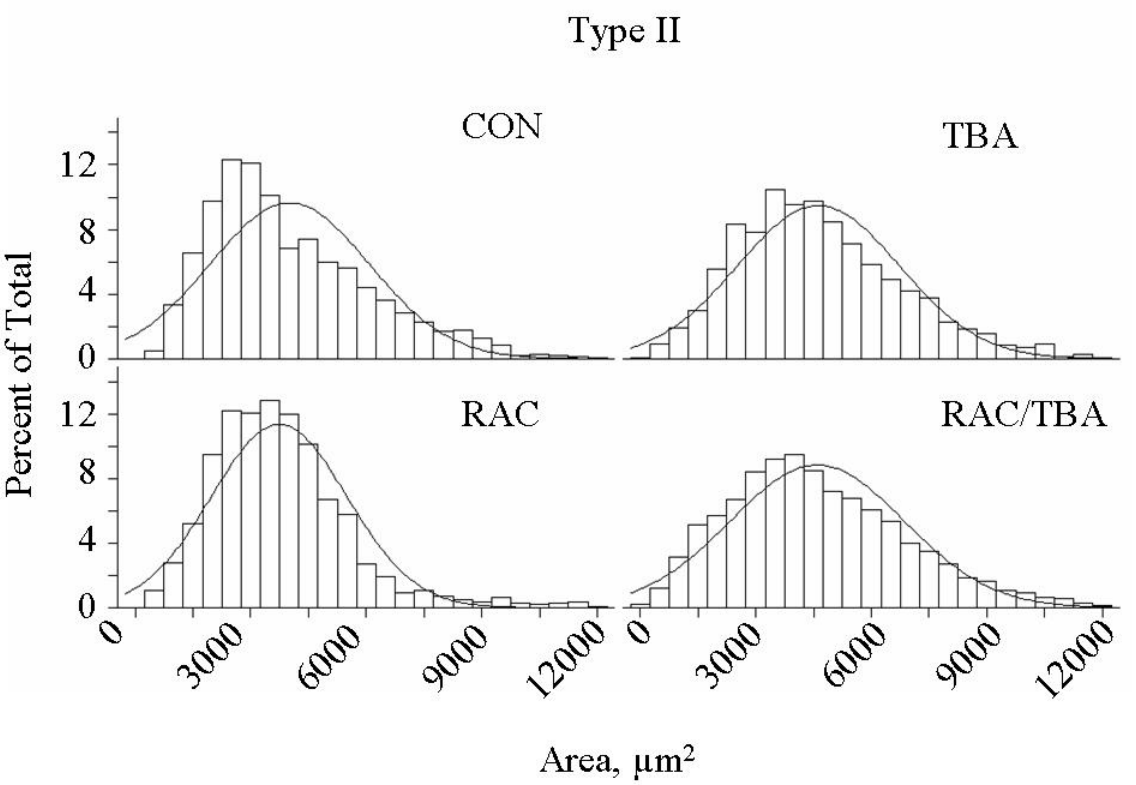

Figure 3-4. Histograms of LM fiber cross-sectional areas of all type IIA fibers sampled from (Control) control diet fed cows, (RAC) control diet + RAC fed cows, (TBA) control diet + implant fed cows, and (RAC/TBA) control diet + RAC + implant fed cows.

CHAPTER 4
DIFFERENTIAL RESPONSE OF CULL COW MUSCLES TO THE HYPERTROPHIC ACTIONS OF RACTOPAMINE-HCl

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Introduction

Ractopamine-HCl (**RAC**) is a beta-adrenergic agonist (**βAR**) approved for use in beef cattle in the United States. Beta-adrenergic agonists improve feedlot performance in growing beef steers and heifers as evidenced by increased average daily gain and feed efficiency (Avendano-Reyes et al., 2006; Walker et al., 2006). However, cattle fed RAC demonstrate only modest improvements in carcass traits (Gruber et al., 2007). Heavier hot carcass weights (**HCW**) with no changes in loin eye area (**LEA**), yield grade or measures of fat deposition were observed in steers fed RAC (Winterholler et al., 2007). Others report an increase in both HCW and LEA in RAC-fed steers (Gruber et al., 2007). A similar tempered response is evident in heifers. Ractopamine augmented heifer feedlot performance measures, HCW, LEA and yield grade (Sissom et al., 2007). By contrast, Walker (2006) found no improvement in HCW, LEA or yield grade in heifers receiving RAC. The disparity in RAC effects in fed cattle remains unresolved.

Individual tissue responses to RAC are a consequence of βAR isoform expression and numbers. Swine adipocytes express an equal percentage of β_1 and β_2 ARs while muscle fibers express predominately the β_2 AR (Liang and Mills, 2002; Sillence et al., 2005; Spurlock et al., 1994). Interestingly, βAR density is reduced in backfat depots in pigs fed RAC but not in skeletal muscle (Spurlock et al., 1994). Down-regulation of βAR may account for the loss of a lipolytic effect over time of RAC feeding. In cattle, transcripts for all three βAR isoforms are

present in skeletal muscle (Walker et al., 2007). RAC supplementation to steers and heifers causes a reduction in β_2AR mRNA with no effect on either β_1 or β_3-*AR* expression (Sissom et al., 2007; Winterholler et al., 2007). The ability of RAC supplementation to down-regulate muscle β_2−ARs in cull-cows is unknown. Due to the subtle improvements in lean deposition in cattle, a more thorough investigation of RAC effects on muscles of varying fiber type composition is required. The objective of this study was to examine the effects of varying concentrations of RAC on fiber hypertrophy and βAR gene expression from muscles located in the fore- and hindquarter of cull beef cows.

Materials and Methods

Animals and Diets

Eighty eight Beefmaster and Angus-type cull cows were stratified by breed and weight to one of four RAC supplementation treatments. Cows consumed a concentrate diet (Table 4-1) *ad libitum* for 54 days prior to harvest. Ractopamine-HCl (0, 100, 200, and 300 $mg \cdot hd^{-1} \cdot d^{-1}$) was supplemented during the final 28 days on feed as a pelleted Type B premix that consisted of wheat middlings (97.6%) and ractopamine-HCl (2.4%) (Optaflexx 45; Elanco Animal Health, Greenfield, IN). The appropriate concentration of RAC of each treatment group premix was formulated based on a projected DMI of approximately 13.6 $kg \bullet hd^{-1} \bullet d^{-1}$. The initial body weights of the four treatment groups were 426.3 kg, 436.9 kg, 418.8 kg and 439.0 kg for the 0, 100, 200, and 300 groups, respectively. At the end of the feeding portion cows weighted 490.0 kg, 483.1 kg, 466.7 kg, and 497.8 kg for the 0, 100, 200, and 300 groups, respectively.

Harvesting and Sample Collection

Cows were slaughtered at a commercial USDA-inspected facility. Within 60 minutes of exsanguination, portions of the *longissimus* (**LM**) and *semimembranosus* (**SM**) muscles from

four randomly selected animals per group (n = 16) were collected and frozen in liquid nitrogen for RNA extraction. Twenty-four hours postmortem, whole muscles of the LM, SM, *Infraspinatus* (**INF**), and *Vastus lateralis* (**VL**) were transported to the University of Florida Meats Laboratory. Two 1 cm^3 portions of each muscle from 10 randomly selected cows per group (n = 40) were suspended in OCT tissue freezing medium (Fisher Scientific, Hampton, NH), frozen by submersion in super-cooled isopentane, and stored at -80°C.

Immunohistochemistry

The methods used by Gonzalez et al. (2007) were followed for immunohistochemical staining. Briefly, two 12 micrometer serial cryosections were collected on frost resistant slides (Fisher Scientific, Hampton, NH). Non-specific antigen sites were blocked with 5% horse serum in phosphate buffered saline (**PBS**). Cryosections were incubated in primary antibodies for 60 minutes at room temperature. Primary antibodies and dilutions were α-dystrophin (Abcam, Cambridge, MA) 1:50, myosin heavy chain type I (BAD.5, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA) hybridoma supernatant, and myosin heavy chain type IIA (SC.71, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA) hybridoma supernatant. After washing with PBS, tissues were incubated in secondary antibodies for 45 minutes at room temperature. Labeled secondary antibodies included rabbit anti-mouse AlexaFluor® 568 (Invitrogen, San Diego, CA) for α-dystrophin detection and goat anti-rat biotin (Vector Laboratories, Burlingame, CA) followed by steptavidin AlexaFluor® 488 (Invitrogen, San Diego, CA) for myosin heavy chain isoform detection. Hoechst 33245 was used to detect nuclei. After a final PBS wash, slides were cover-slipped and fluorescence was visualized using an Eclipse TE 2000-U microscope (Nikon, Lewisville, TX) equipped with an X-Cite 120 epifluorescence illumination system (EXFO, Mississauga, Ontario, Canada). Images were captured using a DXM 1200F digital camera (Nikon, Lewisville, TX) and analyzed for

individual muscle fiber area and diameter using the NIS-Elements software (Nikon, Lewisville, TX). For each animal, a minimum of 1,000 fibers were measured and analyzed. The region constrained by α-dystrophin immunostaining defined individual fibers for cross-sectional area (**CSA**) and diameter measurement. Fiber associated nuclei (**FAN**) were identified as Hoechst 33245 labeled nuclei lying adjacent to the α-dystrophin border. The number of fibers located within each micrograph was counted to determine the number of nuclei per fiber CSA.

RNA Extraction and Real-Time PCR Analysis

Five hundred milligrams of muscle was homogenized in 10 mL STAT-60 (Tel-Test Inc., Friendswoods, TX) with a mechanical tissue disruptor. Two mL of chloroform was added and the upper aqueous layer containing nuclei acids was collected by centrifugation. RNA was precipitated by isopropanol and centrifugation. The nucleic acid pellet was washed with 70% ethanol and air-dried. Pellets were resuspended in sterile-filtered, double distilled water and further purified using the PureLink™ Micro-to-Midi Total RNA Purification System (Invitrogen, San Diego, CA). Purity of the RNA was evaluated by spectroscopy with all samples exhibiting an OD260:280 greater than 1.9. Integrity of RNA was verified by the presence of intact ribosomal RNA bands following electrophoresis through ethidium bromide impregnated agarose gels. Aliquots of RNA were stored at -80 C.

One μg of total RNA was treated with RNase-free DNAse (Promega, Madison, WI) to remove trace genomic DNA contamination. Subsequently, the RNA was reverse transcribed with MMLV-Reverse Transcriptase (Ambion, Austin, TX) and random hexamers at 42°C for 60 minutes. cDNA from 50 ng of RNA was amplified with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) and the appropriate forward and reverse primers (20 pM; Table 4-2) in an ABI 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA). Thermal cycling parameters included a denature step of 95°C for 10 minutes and 40 cycles of

15s at 95.0ºC and 1 minute at 55.0ºC. A final dissociation step included 95°C for 15 seconds, 60°C for 30 seconds, and 95°C for 15 seconds. Following the procedures of Castellani et al. (2004) serial dilutions of pooled samples were used to generated standard curves to ensure generation of Ct values were within the linear range of amplification.

Statistics

Use of carcass as the experimental unit and data was statistically analyzed similar to Wheeler et al. (1990) and Gonzalez et al. (2007). Data for gene expression and FAN numbers were analyzed as a split-plot design using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, 2002). The whole plot consisted of breed type, RAC treatment, and whole plot error. The whole plot error consisted of breed type x RAC treatment. The subplot was muscle, breed type x muscle, RAC treatment x muscle interaction, and the subplot error. The subplot error was comprised of the three-way interaction between breed type, RAC treatment, and muscle. Muscle fiber CSA and diameter data was analyzed using a split-split-plot design. The whole plots and subplots were the same as the split-plot analysis. The sub-subplot consisted of muscle fiber type and the remaining interactions. The four factor interaction between breed type, RAC treatment, muscle, and fiber type were used as the sub-subplot error. Pair-wise comparisons between the least square means of the factor levels were computed by using the PDIFF option of the LSMEANS statement.

Results

Cryosections from each of the four muscles were immunostained for MyHC type I and IIA isoforms. Morphometrics for type I and II fibers from the LD, VL, SM and INF were measured (Table 4-3). Ractopamine fed at a rate of 100 or 300 $mg \cdot hd^{-1} \cdot d^{-1}$ (RAC-100 and RAC-300, respectively) altered ($P < 0.05$) the fiber type composition in all muscles examined. Ractopamine fed at 200 $mg \cdot hd^{-1} \cdot d^{-1}$ (RAC-200) caused an increase ($P < 0.05$) in the percentage

of type IIA fibers in the LD, SM and VL. By comparison to the LD, the SM and VL had the largest fiber type distribution shifts. By contrast, a shift toward more ($P < 0.05$) type I fibers in the INF was measured in cull cows fed RAC-200 and 300. RAC-200 tended to increase ($P = 0.14$) the diameter and CSA of the type I fibers within the VL and significantly increase ($P = 0.05$) type IIA fibers within the SM. An increase ($P < 0.05$) in the dimensions of both type I and IIA fibers were observed within the INF of cull cows fed RAC-100; no changes ($P > 0.05$) in fiber CSA or diameter were observed within the LD, SM or VL.

As reported previously, RAC-200 does not increase the calculated LD myonuclear domain (Gonzalez et al., 2007). Fewer ($P < 0.05$) myonuclei per fiber were observed in the LD and VL of cull cows fed RAC-200; no changes ($P > 0.05$) were found in the INF or SM (Table 4-4). Cows fed RAC-100 contained fewer ($P < 0.05$) FAN in the INF, LD and VL than controls. RAC-300 cull cows contained fibers with fewer FAN within the LD and VL.

Semi-quantitative RT-PCR indicated that RAC supplementation at any level did not change ($P > 0.05$) the amount of detectable β_2-AR, MyHC type I, IIA or IIX mRNA in the LD (Table 4-5). Compared to controls, RAC decreased ($P < 0.05$) β_2-AR, MyHC type I and IIX mRNA content in the SM when fed at 100 $mg \cdot hd^{-1} \cdot d^{-1}$ (Table 4-5). RAC supplementation at 200 $mg \cdot hd^{-1} \cdot d^{-1}$ tended to increase both β_2-AR and MyHC IIX mRNA abundance in the SM.

Discussion

RAC supplementation to growing cattle offers an advantageous improvement in performance parameters including increased average daily gains and lower feed to gain ratios (Walker et al., 2007). However, these performance measures translate into only modest improvements in carcass traits. The tempered responses to RAC are further confounded by sex. Heifers fed RAC (200 $mg \cdot hd^{-1} \cdot d^{-1}$) require less feed per unit of weight gain but do not differ from

control heifers with regard to ADG, DMI or carcass parameters (Sissom et al., 2007; Walker et al., 2006). In a similar manner, non-implanted heifers fed both 200 and 300 $mg \cdot hd^{-1} \cdot d^{-1}$ of RAC during the final 28 days of a 42 day feeding trial demonstrated no improvements in live performance parameters or carcass weight (Quinn et al., 2008). The beef cull cows fed RAC in the current study demonstrated no improvements in either feedlot performance (Carter and Johnson, 2007) or carcass traits (Dijkhuis et al., 2008). Thus, the lack of an improved performance response is not due to the age of our animals but may be related to animal gender.

Ractopamine concentration was sufficient to cause a biological response in cull cows as evidenced by an increase in muscle fiber CSA (Gonzalez et al., 2007). However, larger muscle fiber CSA stimulated by RAC fed at the manufacturer's recommended dose (200 $mg \cdot hd^{-1} \cdot d^{-1}$) does not translate into larger REA (Carter et al., 2006). The present study addressed the possibility that a higher concentration of RAC may be required to elicit a global improvement on muscle cell size in aged cows. Ractopamine supplementation at a rate of 300 $mg \cdot hd^{-1} \cdot d^{-1}$, a concentration within the recommended feeding guideline, provoked a response no different than the conventional 200 $mg \cdot hd^{-1} \cdot d^{-1}$ feeding rate. The measured CSA of LD type I and II fibers did not differ between controls, RAC-100 or RAC-200. The shift in fiber type in RAC treated animals indicates that the feed additive is bioactive. Assuming an equivalent rate of absorption and cellular delivery, no change in fiber morphometrics would suggest that the limitation to further increases in muscle growth are not a consequence of insufficient RAC concentration.

The lack of a robust increase in fiber size is not likely attributable to low numbers of β_2AR. β_2AR is the preferred receptor for RAC in swine tissues (Mills et al., 2003) and the receptor is present in skeletal muscles of cattle (Bridge et al., 1998). Our results indicate that the LD and SM transcribe the β_2 adrenergic receptor gene and transcript abundance is not diminished in

response to RAC. Indeed, SM β_2AR mRNA levels tend to be increased by RAC-200, as reported by others (Sissom et al., 2007; Winterholler et al., 2007). Because receptor protein expression was not determined in any of these studies, it remains possible that female cattle are refractile to the positive effects of RAC at the cellular level due to low abundance βAR numbers and/or deficits in the signal transduction system.

As reported in swine (Depreux et al., 2002; Gunawan et al., 2007), RAC initiated a shift in fiber types from slow to fast. A reduction in the percentage of type I slow fibers is evident in the LD, SM and VL with the relative decline between muscles variable. In the LD, RAC-100 is sufficient to elicit a reduction in the percentage of type I fibers with no further decline with increased RAC consumption. By contrast, the SM and VL are highly variable in their response to RAC. In both muscles, RAC-200 elicited the greatest effect with approximately 30% of the type I fibers transitioning to a fast isoform. In the SM, the increased number of type II fibers is associated with greater CSA and a tendency toward higher amounts of MyHC-IIX mRNA. Further increases in RAC did not exacerbate the shift but caused a dampened response. The slight increase in type I fibers in the SM and VL of cows receiving RAC-300 versus RAC-200 may indicate a down-regulation of key mediator(s) of RAC effects. The identity of the intracellular mediators of RAC signals in bovine muscle fibers is unknown at this time.

The divergence in responses to RAC amongst muscles is further exemplified by fiber type shifts in the INF. The INF, a forelimb muscle, is nearly a 50:50 mix of slow and fast fibers. RAC-100 caused a reduction in the numbers of oxidative type I fibers, as expected. However, RAC-200 behaved in the opposite manner with a significant increase in the numbers of slow fibers. RAC-300 did not differ from RAC-200 in these measured parameters. Feeding RAC-100 also caused a 65-70% increase in the size of the INF muscle fiber independent of metabolic

enzyme classification. The unexpected shift from fast to slow fiber types also was noted in the SM of cows fed RAC-100 without a change in CSA. Interestingly, the larger SM fiber CSA in RAC-100 cows is associated with a reduction in MyHC gene expression. The dichotomy of larger fibers with less MyHC mRNA suggests that the increased size may reflect a reduction in protein degradation or prolonged half-life of the contractile protein. At the very least, these results underscore the complexity of RAC effects in cattle.

The results presented in this study support our previous work demonstrating that fiber size increases without an increase in myonuclei numbers (Gonzalez et al., 2007). Each fiber contains hundreds of myonuclei, and the ratio of nuclei/cytoplasm, or myonuclear domain, must remain constant (Aberle et al., 2001). O'Connor and Pavlath (2007) described muscle fiber growth as having an initial phase characterized by enhanced transcription and translation, leading to increased protein accretion and a small expansion of the myonuclear domain. Following this initial growth, fusion of satellite cells must occur to combat the threshold or ceiling established by myonuclear domain and facilitates additional increases in fiber CSA. However, O'Connor et al. (2007) concluded that increases in nuclear content are not needed to induce skeletal muscle growth, and strong evidence is provided by the ability of β-agonists to promote protein synthesis without the addition of myonuclei. The mechanism underlying the increase in muscle fiber size in response to RAC has been linked to altered protein turnover. In pigs, poultry, lambs, and cattle, numerous β-agonists including RAC, cimaterol, and clenbuterol increased skeletal muscle hypertrophy in the absence of increases in either DNA content or myonuclear number (Beermann et al., 1987; Dunshea et al., 1993; Gwartney et al., 1992; Smith et al., 1987). Pigs fed RAC demonstrate an increase in fractional protein synthesis rates (Dunshea et al., 1993; Dunshea et al., 1998; Williams et al., 1994). To date, direct measurement of protein turnover rates in

cattle receiving RAC have not been reported. Thus, in cattle RAC likely stimulates muscle fiber growth by a change in protein synthesis and/or degradation rates.

Conclusion

Ractopamine supplementation to cattle causes a biological effect on muscle fiber isoform distribution and size at concentrations ranging from 100 to 300 $mg \cdot hd^{-1} \cdot d^{-1}$. Cull beef cows fed RAC-100 responded in a manner similar to conventional RAC-200 as measured by a shift in muscle fiber isotypes. Interestingly, the lower concentration of RAC improved fiber size only in the INF, a muscle characterized by a higher proportion of red fibers. Cull cow feeding programs may consider supplementing RAC-100 as a means of adding value to cuts within the chuck, such as the INF.

Table 4-1. Composition of basal diet[1]

Item	Amount, percent of DM
Soybean hulls	21.1
Citrus pulp	19.7
Cracked corn	14.4
Wheat middlings	14.2
Cottonseed hulls	12.7
Cottonseed meal	7.0
Liquid molasses	7.0
Vitamins and minerals	2.1
Tallow	1.3
Urea	0.4

[1] Diet designed to meet the nutrient requirements of a non-pregnant, non-lactating, beef cow predicted to gain 2.06 kg/d and provided 87.6% DM, 14% crude protein (DM basis), and 79.5% TDN (Abney et al., 2007). An ionophore [Rumensin 80 (monensin granulated); Elanco Animal Health, Greenfield, IN] was also included in the basal diet at the formulated rate of 22 mg/kg of feed.

Table 4-2. Sequence of bovine-specific PCR primers used for determination of the expression of mRNA for β_1- and β_2-adrenergic receptors and myosin heavy chain isoforms

Gene of interest	Primer
18S	
Forward	5′-GTAACCCGTTGAACCCCATT-3′
Reverse	5′-CCATCCAATCGGTAGTAGCG-3′
β_2	
Forward	5′-TCATGTCGCTTATTGTCCTGG-3′
Reverse	5′-TCATGTCGCTTATTGTCCTGG-3′
Myosin Heavy Chain	
MYO2[a]	5′-ATCCAGGCTGCGTAACGCTCTTTGAGGTTGTA-3′
MYO111[b]	5′-CACTTGCTAACAAGGACCTCTGAGTTC-3′
MYO209[c]	5′-CTTTCCTCATAAAGCTTCAAGTTCTGACC-3′
MYO405[d]	5′-TGCTGCTCTCAGGCCCCTGCCACCTT-3′

[a] Common reverse sequence used for all three myosin heavy chain isoforms (Tanabe 1998).
[b] Forward sequence used for detection of MyHC IIA (Tanabe et al., 1998).
[c] Forward sequence used for detection of MyHC IIX (Tanabe et al., 1998).
[d] Forward sequence used for detection of MyHC I (Tanabe et al., 1998).

Table 4-3. Least squares means of muscle fiber myosin heavy chain isoform distribution, cross-sectional area and diameter from four muscles of cull-cows fed three levels of ractopamine-HCl

		Type I Fiber			Type IIA Fiber		
Muscle[1]		Percentage	Area (μm^2)	Diameter (μm)	Percentage	Area (μm^2)	Diameter (μm)
INF							
	CON	51.1[a]	4545 ± 384[a]	74 ± 4[a]	48.9[a]	2554 ± 384[a]	56 ± 4[a]
	100	45.7[c]	6432 ± 385[b]	88 ± 4[b]	54.3[c]	3871 ± 385[b]	68 ± 4[b]
	200	54.3[b]	3955 ± 384[a]	69 ± 4[a]	45.7[b]	2617 ± 384[a]	55 ± 4[a]
	300	54.8[b]	4927 ± 384[a]	77 ± 4[a]	45.2[b]	3100 ± 384[a,b]	61 ± 4[a]
LD							
	CON	34.4[a]	2109 ± 385	49 ± 4	65.6[a]	3124 ± 384	60 ± 4
	100	32.6[b]	2753 ±385	57 ± 4	67.4[b]	3116 ± 384	61 ± 4
	200	32.2[b]	2833 ± 385	58 ± 4	67.8[b]	3473 ± 384	64 ± 4
	300	32.6[b]	2090 ± 385	50 ± 4	67.4[b]	3591 ± 384	65 ± 4
SM							
	CON	34.0[a]	1980 ± 385	49 ± 4	66.0[a]	3127 ± 354[a]	61 ± 4[a]
	100	39.5[b]	2067 ± 386	50 ± 4	60.5[b]	3820 ± 384[a,b]	67 ± 4[a,b]
	200	23.6[c]	2368 ± 385	54 ± 4	76.4[c]	4175 ± 384[b]	71 ± 4[b]
	300	27.5[d]	1937 ± 385	47 ± 4	72.5[d]	3674 ± 384[a,b]	64 ± 4[a,b]
VL							
	CON	35.3[a]	2284 ± 384[x]	52 ± 4[x]	64.7[a]	3046 ± 384	60 ± 4
	100	31.0[b]	2722 ± 385[x,y]	56 ± 4[x,y]	69.0[b]	3554 ± 384	65 ± 4
	200	24.6[c]	3044 ± 385[y]	61 ± 4[y]	75.4[c]	3658 ± 384	66 ± 4
	300	29.1[d]	2222 ± 385[x]	51 ± 4[x]	70.9[d]	3322 ± 384	62 ± 4

[1] Muscles: INF = *Infraspinatus*; LD = *Longissimus dorsi*; SM = *Semimembranosus*; VL = *Vastus lateralis*. Treatments: CON = 0 $mg \cdot hd^{-1} \cdot d^{-1}$; 100 = 100 $mg \cdot hd^{-1} \cdot d^{-1}$; 200 = 200 $mg \cdot hd^{-1} \cdot d^{-1}$; 300 = 300 $mg \cdot hd^{-1} \cdot d^{-1}$ of ractopamine-HCl supplemented during the final 30 days of feeding.

[a,b] Means within a muscle and column with different letters are significantly different ($P < 0.05$).

[x,y] Means within a muscle and column with different letter are different ($P < 0.15$).

Table 4-4. Myonuclei per fiber cross-section from cull-cows fed four different levels of ractopamine-HCl

Muscle[1]	Fiber-associated nuclei
INF	
0	1.87 ± 0.05[a]
100	1.70 ± 0.06[b]
200	1.78 ± 0.06[a,b]
300	1.81 ± 0.06[a,b]
LD	
0	1.35 ± 0.06[a]
100	1.07 ± 0.06[b]
200	1.15 ± 0.06[b]
300	1.13 ± 0.05[b]
SM	
0	1.55 ± 0.06
100	1.64 ± 0.07
200	1.68 ± 0.06
300	1.52 ± 0.06
VL	
0	2.06 ± 0.06[a]
100	1.50 ± 0.07[b]
200	1.82 ± 0.06[c]
300	1.86 ± 0.06[c]

[1] Muscles: INF= *Infraspinatus*; LD = *Longissimus dorsi*; SM = *Semimembranosus*; VL= *Vastus lateralis*. Treatments: CON = 0 mg·hd^{-1}·d^{-1}; 100 = 100 mg·hd^{-1}·d^{-1}; 200 = 200 mg·hd^{-1}·d^{-1}; 300 = 300 mg·hd^{-1}·d^{-1} of ractopamine-HCl supplemented during the final 30 days of feeding.

[a-c] Means within a muscle and column with different letters are significantly different ($P < 0.05$).

Table 4-5. Real time PCR ΔCt values for β_2-adrenergic receptor, myosin heavy chain isoform expression from the *Longissimus dorsi* and *Semimembranosus* of cull-cows fed four levels of ractopamine-HCl[1]

Muscle[2]	β_2-receptor ΔCt	Type I ΔCt	Type IIA ΔCt	Type IIX ΔCt
Longissimus dorsi				
0	10.24	11.71	14.55	20.95
100	10.11	11.50	13.10	22.10
200	9.84	9.94	12.27	20.19
300	10.62	10.34	12.74	21.55
Semimembranosus				
0	$10.35^{a,x}$	9.79^{a}	11.12^{x}	$13.67^{a,x}$
100	13.11^{b}	$13.17^{b,x}$	14.59^{y}	21.01^{b}
200	$8.80^{a,y}$	9.31^{a}	11.22^{x}	$10.86^{a,y}$
300	$8.63^{a,y}$	$10.39^{a,y}$	11.96^{x}	12.57^{a}
SEM	0.88	1.27	1.35	1.11
Slope[3]	-3.10	-3.45	-3.57	-3.22

[a,b] Means within a muscle and column with different letters are significantly different ($P < 0.05$).

[x-y]Means within a muscle and column without common superscript tend to differ ($P < 0.10$).

[1] Bovine 18S gene expression used as house keeping gene for computation of ΔCt values. 18S gene expression did not differ between treatments of both muscle groups. ΔCt = Ct gene of interest – Ct 18S.

[2] Treatments: CON = 0 mg·hd^{-1}·d^{-1}; 100 = 100 mg·hd^{-1}·d^{-1}; 200 = 200 mg·hd^{-1}·d^{-1}; 300 = 300 mg·hd^{-1}·d^{-1} of ractopamine-HCl supplemented during the final 30 days of feeding.

[3]Slope of standard curve of primers sequences used for real-time PCR analysis. Bovine 18S gene slope = -3.22.

CHAPTER 5
EFFECT OF RACTOPAMINE-HCl ON THE FIBER TYPE DISTRIBUTION AND SHELF-LIFE OF SIX MUSCLES OF STEERS

Introduction

Feeding ractopamine-HCl (**RAC**; 200 $mg \cdot hd^{-1} \cdot d^{-1}$) to steers influences live and carcass performance in a positive manner. Schroeder et al. (2005a) reported that RAC improves both average daily gain and gain to feed ratio by 26%, and Winterholler et al. (2007) reported that total gain can significantly improve by 6 percent. RAC increases hot carcass weight and ribeye area, decreases fat, and increases dressing percentage by as much as 3.6% (Schroeder et al., 2005b; Winterholler et al., 2008).

Color represents the single most important visual component that determines if a consumer will purchase a meat product (Hedrick et al., 1994). Several biochemical and physical factors affect meat color stability. The metmyoglobin reducing system relies heavily on NADH to chemically reduce metmyoglobin to deoxymyoglobin (Mancini and Hunt, 2005) depressing discoloration and promoting color stability (McKenna et al. 2005). NADH content in meat is determined by the energy metabolism profile of the individual muscle fibers (Howlett and Willis, 1998). NADH content is higher in type I slow fibers than in type II fast twitch fibers. A loss of type I fibers may cause a reduction in metmyoglobin reducing activity thus, negatively impacting meat color. In both swine (Aalhus et al., 1992) and cattle (Gonzalez et al., 2007; 2008), data show that RAC can induce a fiber type shift from type I slow-twitch to type II fast-twitch fibers by as much as 30%. Therefore, the objective of this study was to evaluate the effects of RAC on MyHC isoform distribution and shelf-life properties of muscles of the loin and round.

Materials and Methods

Animals and Pre-Harvest Diets

This experiment was approved by the University of Florida Institutional Animal Care and Use Committee. Thirty-four crossbred steers were selected from steers housed at the University of Florida Beef Teaching Unit. Upon selection, steers were separated into four harvest groups (three groups of eight and one group of ten). All cattle were administered a Ralgro implant (36 mg Zeranol) followed by a Revalor-S implant (120 mg trenbolone acetate and 24 mg estradiol). Within each harvest group, steers were stratified by breed type and separated into two pens so that initial pen weight and visual backfat thickness were similar. Pens were established two weeks prior to RAC supplementation to allow establishment of herd dynamics.

Steers were fed daily a concentrate diet consisting of 85% corn, 7.5% cottonseed hulls, and 7.5% commercially produced protein pellet. All steers were supplemented with a hand mixed top dress (0 $mg \bullet hd^{-1} \bullet d^{-1}$ of RAC; dehydrated alfalfa meal, corn meal, calcium carbonate, rice hulls, soybean oil, and mineral oil) at a rate of 0.45 kg per head per day for two weeks prior to the RAC supplementation period. Subsequently, the treatment pens received 0.91 kg per head per day of hand mixed top dress designed to provide 200 $mg \bullet hd^{-1} \bullet d^{-1}$ of RAC (Elanco Animal Health, Greenfield, IN) for 28 days prior to harvest. Control animals were fed an equivalent amount of top dress. The top dress provided 11% crude protein, 5% crude fat, and 16% crude fiber.

Harvesting and Sample Collection

Steers were harvested at the University of Florida Meat Laboratory under Federal Inspection following the Humane Methods of Slaughter Act of 1978. Seventy-two hours postmortem, the bone-in strip loin, knuckle, and top round were excised from the right side of each carcass. Whole muscles were removed from the subprimals including the *Longissimus*

lumborum (**LL**) from the bone-in strip loin; the *Adductor* (**ADD**), *Gracilis* (**GRA**), and *Semimembranosus* (**SM**) from the top round; and the *Rectus Femoris* (**RF**) and *Vastus lateralis* (**VL**) from the knuckle. A 1 cm^3 portion was removed from each muscle, frozen in OCT tissue freezing medium (Fisher Scientific, Hampton, NH), and stored at -80°C for immunohistochemical analysis. Whole muscles were placed in heat shrink vacuum bags (B2570; Cryovac, Duncan, SC) and vacuum packaged using a Multivac C500 (Multivac, Inc., Kansas City, MO). Muscles were wet aged for 13 days postmortem at 1 ± 3°C.

Immunohistochemistry

The methods used by Gonzalez et al. (2007, 2008) for immunohistochemical staining were followed with slight modifications. Twelve micrometer cryosections from each muscle were collected on frost resistant slides (Fisher Scientific, Hampton, NH). Non-specific antigen sites were blocked with 5% horse serum in phosphate buffered saline (PBS). Cryosections were incubated in primary antibodies for 60 minutes at room temperature. Primary antibody solution consisted of anti-myosin heavy chain type I (BAD.5, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA) hybridoma supernatant with anti-α-dystrophin (1:50; Abcam, Cambridge, MA). After washing with phosphate buffered saline (**PBS**), cryosections were incubated in goat anti-rat biotin (Vector Laboratories, Burlingame, CA) for 20 minutes at room temperature. After washing with PBS, cryosections were incubated in rabbit anti-mouse AlexaFluor® 568 (Invitrogen, San Diego, CA) and steptavidin AlexaFluor® 488 (Invitrogen, San Diego, CA) for 45 minutes to detect dystrophin and MyHC type I, respectively. After a final PBS wash, slides were visualized using an Eclipse TE 2000-U microscope (Nikon, Lewisville, TX) equipped with an X-Cite 120 epifluorescence illumination system (EXFO, Mississauga, Ontario, Canada). Photomicrographs were captured using a Photometrics Cool Snap EF digital camera (Nikon, Lewisville, TX) and analyzed for individual muscle fiber cross-sectional area

(CSA) using the NIS-Elements software (Nikon, Lewisville, TX). For each animal, a minimum of 500 fibers were measured and analyzed. All fibers not labeled as MyHC type I were assumed to be type II fast-twitch. The region constrained by α-dystrophin immunostaining defined individual fibers for CSA measurement.

Steak Cutting, Packaging, and Display

Following aging, muscles were removed from their vacuum bags and cut into six 1.27 cm steaks. Steaks were cut from the same end of each muscle, perpendicular to the orientation of the muscle fibers. The first five steaks were used for reduction of nitric oxide metmyoglobin (**NOM**) analysis at days zero, one, two, three, and four. The sixth steak was used for daily visual panel evaluations and day five reduction of NOM analysis. Steaks from the LD and VL were placed on 17S Styrofoam trays (Genpack, Glens Falls, NY), steaks from the ADD, GRA, and VL were placed on 1S Styrofoam trays (Genpack, Glens Falls, NY), and steaks from the SM were placed on 10S Styrofoam trays (Genpack, Glens Falls, NY). Each tray contained a Dri-Loc 40 gram white meat pad (Sealed-Air Corporation, Elmwood Park, NJ) and was overwrapped with polyvinylchloride film (23,250 cc O_2/m^2/24h °C/%RH). Steaks were displayed in a Hill (Hill Refrigeration Div., Trenton, NJ) coffin-style retail case at 2 ± 3°C for five days. Cases were illuminated with GE T8 Linear Fluorescent lamps (2800 lumens, 4100 K; General Electric Company, Fairfield, CT) that emitted a case average of 106.7 footcandle with a 12 hour on 12 hour off lighting schedule. Steaks were rotated daily to compensate for uneven temperature and light distribution within the case.

Nitric Oxide Metmyoglobin Reducing Analysis

The procedures of Watts et al. (1966), Sammel et al. (2002), and McKenna et al. (2005) were followed with minor adjustments. Each day, samples of each steak measuring 5 cm x 5cm x 1.27 cm were placed in 400 mL Pyrex beakers (Corning Inc., Acton, MA). Samples were

oxidized in 50 mL of 0.3% sodium nitrite (Fisher Scientific, Hampton, NH) at 25 ± 2°C for 30 minutes. The samples were removed from the sodium nitrite solution, blotted of excess solution, and vacuum packaged in FoodSaver (Jarden Corp., Rye, NY) 27.94 cm bags with an oxygen transmission rate of 6.7 cc/m^2/24 hr/23°C/0% RH. Reflectance measurements ranging from 400-700 nm were taken every 30 minutes for two hours using a HunterLab MiniScan XE (HunterLab, Reston, VA) spectrophotometer with a 2.54 cm aperture. Spectrophotometric measurements were captured using illuminant A and 10° standard observer. Before each data collection period, the MiniScan was calibrated on both a black and white tile. Spectral data collected at 525 nm and 572 nm were used to calculate metmyoglobin percentage following American Meat Science Association (AMSA) procedures (2003). Nitric oxide metmyoglobin reducing ability was calculated as (observed decrease in metmyoglobin concentration ÷ initial metmyoglobin concentration) × 100.

Subjective and Objective Color Analysis

A six to eight member experienced panel evaluated steaks for beef lean color (8 = extremely bright cherry red; 1 = extremely dark red), fat color (5 = yellow; 1 = white), and surface discoloration (7 = total [100%] discoloration; 1 = no [0%] discoloration) daily for 5 days. Objective color measurements of these samples were taken using a HunterLab MiniScan XE. Illuminance and aperture settings, as well as calibration procedures, were the same as described above. Spectral reflectance data from 400 to 700 nm was used to calculate ratios of metmyoglobin and oxymyoglobin present on the surface of the steaks, according to AMSA procedures (2003). Following collection of spectral data, absolute L*, a*, b* reflectance data were collected on the same samples. Two measurements per steak to represent the color of the entire steak were averaged for spectral and absolute data.

Statistics

Data were analyzed using carcass as the experimental unit. Reducing activity, objective, and subjective color data was analyzed as a split-plot design with repeated measures. Kill group and treatment was considered the whole plot and muscle was considered the sub-plot. Kill group × treatment was utilized as the random error for the whole-plot, and kill group × the treatment/muscle interaction was considered the random error for the sub-plot. Day was the repeated measure with animal within treatment as the subject. Muscle fiber CSA data was analyzed using a split-split-plot design. The whole plot and subplot variables were the same as in the shelf-life data. The sub-subplot consisted of muscle fiber type and the remaining interactions. Kill group × treatment was considered the random error for the whole-plot, kill group × the treatment/muscle interaction was considered the random error for the sub-plot, and the kill group × the treatment/muscle/fiber type interaction was considered the random error for the sub-sub-plot. All measured variables were analyzed with the PROC MIXED procedure of SAS (SAS Inst. Inc., Carry, NC, 2002). Pair-wise comparisons between the least square means of the factor levels were computed using the PDIFF option of the LSMEANS statement. Fiber frequencies were tabulated and compared by chi-square analysis using PROC FREQ statement of SAS. Treatment group frequencies within a fiber type were compared to one another by a two sample t-test for proportions. Differences were considered significant at an alpha = 0.05 and tendencies at an alpha = 0.10.

Results

Ractopamine supplementation did not affect ($P > 0.05$) the CSA of type I or II fibers in the six muscles analyzed (Table 5-1). Ractopamine supplementation caused a significant type I to type II fiber shift in the ADD ($P = 0.0033$), RF ($P < 0.0001$), LL ($P = 0.0044$), and VL ($P <$

0.0001). The VL and GRA exhibited the largest percentage fiber shift. MyHC distribution within the SM remained unchanged by RAC.

Nitric oxide metmyoglobin reducing ability was not significantly affected ($P > 0.05$) by RAC supplementation in the GRA, RF, SM, or VL. The ability of RAC treated steaks from the ADD to reduce nitric oxide metmyoglobin was greater on day zero ($P = 0.05$) and day one ($P = 0.01$) of the display period, but was not different thereafter. The LL was the only other muscle affected by RAC supplementation. *Longissimus lumborum* NOM reducing ability was significantly greater ($P = 0.02$) in RAC treated steaks on day 2. Ractopamine did not affect metmyoglobin or oxymyoglobin accumulation or L*a*b* color scores (Table 5-2 and Table 5-3).

Visual panel scores of the six muscles indicate that RAC supplementation had little affect on either beef lean color or fat color (Table 5-4). Ractopamine did not affect ($P > 0.05$) surface discoloration scores from d0 to d3 of the display period in the GRA, SM, LL, RF or VL. VL steaks from RAC animals had more surface discoloration on day four ($P = 0.05$) and day five ($P = 0.07$). Similarly, RF and SM steaks from RAC steers exhibited higher surface discoloration ($P = 0.0092$ and $P = 0.04$, respectively) than CON at day five. The ADD steaks discolored rapidly with RAC steaks scoring higher ($P = 0.05$) than CON by day three.

Discussion

The benefits of feeding RAC to cattle on both live and carcass characteristics are well documented (Sissom et al., 2007; Winterholler et al., 2007, 2008). However, little data exists profiling the effects of RAC on steak retail display color stability. The oxidation state of myoglobin, the heme-containing protein that stores oxygen in the muscle, determines the color that a consumer sees. Before myoglobin is exposed to oxygen, it is in the deoxymyoglobin state, which results in a purple color. As the myoglobin is exposed to oxygen, it is converted into oxymyoglobin. Oxymyoglobin results in the typical bright cherry red color that the consumer

desires. As meat ages, oxymyoglobin experiences oxidation and the formation of metmyoglobin occurs (Aberle et al., 2001). Concurrently, lipid oxidation occurs in the intramuscular fat, membrane phospholipids, and intermuscular fat. This oxidative system results in the formation of metmyoglobin in the meat and correlates to the amount of discoloration observed (Faustman and Cassens, 1990). In the retail display case, consumers visually evaluate numerous factors when considering which product to purchase (MacKinney et al., 1966). These factors may include portion size, leanness, ease of preparation, or color. According to Hedrick et al. (1994) and Kropf (1980), color is the single most important visual component that determines if a consumer will purchase a meat product. Therefore, the effect of RAC on metmyoglobin accumulation on the surface of steaks requires attention.

Regardless of treatment, muscles observed in the study have different reducing potential, or ability to maintain this potential, during display. The differences observed in reducing potential translated into differences in a* values and $(K/S)_{572}/(K/S)_{525}$ ratios. From day 0 of the study, the LL and SM displayed the largest percent NOM reducing ability, while the RF exhibited the least. The LL maintained the greatest NOM reducing ability throughout the five day display period. The VL and GRA began display with similar NOM reducing abilities, and the VL was able to maintain the second highest percentage of NOM reduced by the end of the study. The ADD and RF had the worst NOM reducing abilities. The ADD did not possess the capability to maintain NOM reducing ability throughout the 5 day study. Initial percent NOM reduced from this muscle was similar to the GRA or VL, and the ADD ultimately possessed the lowest percentage of any muscle.

Spectral data used to calculate ratios of the accumulation of metmyoglobin and oxymyoglobin indicate that muscles followed patterns similar to those reported in the NOM

reducing data. At the beginning of the display period, all muscles exhibited similar ratios for oxymyoglobin and metmyoglobin accumulation. By the end of the display period, the LL and VL had the least amount of metmyoglobin and subsequently the highest amount of oxymyoglobin. In contrast, the ADD and RF had the lowest ratios and accumulated the highest concentration of metmyoglobin and lowest concentration of oxymyoglobin. The rapid increase in ADD and RF metmyoglobin was apparent by the second day of retail display; similar results were observed with a* values. The LL and SM maintained larger a* values throughout the five day study indicating that muscles were redder in appearance.

These findings agree with McKenna et al. (2005) who found that the LL and VL have the highest and the ADD has the worst NOM reducing ability. However, these researchers found that the RF had greater reducing ability than the SM. $(K/S)_{572}/(K/S)_{525}$ ratios indicated that the LL, VL, and SM were the most resistant to metmyoglobin accumulation, and the RF and ADD accumulated metmyoglobin rapidly. Instrument a* values indicated that the LL was the reddest during display. The SM, VL, and RF maintained similar redness values, and the adductor rapidly lost its red hue. Our results, in agreement with McKenna et al. (2005), demonstrate that muscles degrade in color at different rates and should be marketed accordingly to reduce product losses or devaluation.

Dietzel (1990) reported that supplementation of RAC produced steaks with a brighter cherry red lean color after four days of display when compared to untreated steaks. Additionally, steaks from ractopamine fed steers maintained their overall appearance longer than steaks from non-treated steers. In the current study, spectral and absolute data indicates that RAC supplementation did not affect the color stability or metmyoglobin accumulation in steaks from the six muscles analyzed. More importantly, metmyoglobin accumulation, as measured by

$(K/S)_{572}/(K/S)_{525}$ ratio, was unaffected by RAC supplementation. However, visual panel data indicates that RAC did have a detrimental effect on the beef lean color and surface discoloration scores of several muscles. Ractopamine steaks from the VL were darker on days 3 and 5 of display. In addition, CON steaks from the VL appeared to reach a stable lean color score during the last three days of display, while the RAC steaks continued to become darker. Ractopamine treated VL steaks had more surface discoloration during the last three days of display. Ractopamine steaks from the GRA, RF, and SM also demonstrated greater discoloration on day 5 of display. Therefore, while objective measures of color were unable to detect treatment differences, visual panelists observed differences that a consumer may also detect. In agreement with our findings, Neill et al. (2008) found that steaks originating from the knuckle of implanted cull cows fed zilpaterol-HCl were darker and had more discoloration on day 5 of display than cows that were grass fed, concentrate fed, and concentrate fed with the inclusion of zilpaterol.

The decrease in shelf-life witnessed in this study may be attributed to a physiological effect commonly observed when feeding beta-agonists, a shifting of MyHC isoforms. In swine, RAC increases the percentage of type IIB fibers at the expense of type IIA and IIX fibers (Depreux et al., 2002; Guanawan et al., 2007). In older female cattle, RAC included in a high concentrate feeding program can shift MyHC isoforms from type I to type IIA, and from type IIA to type I by as much as 30% depending on the muscle evaluated (Gonzalez et al., 2007, 2008). In the steers evaluated in the current study, all the muscles observed had RAC induced MyHC fiber type shifts except the SM. The lack of fiber shifting in the SM is surprising since the SM had one of the largest MyHC isoform shifts in RAC supplemented cull cows (Gonzalez et al., 2008). In the current study, the VL and GRA had the largest percent shift with 21% and 11% of their type I fibers shifting to type II fibers, respectively. This isoform shift in the VL is in line with

previous report (Gonzalez et al, 2008). The ADD and LD demonstrated 7% and 6% type I to type II shift, respectively. In contrast, the RF had 14% increase in type I fibers at the expense of type II fibers. The shifting of type II fibers to type I fibers seen in the RF was also seen in the infraspinatus of cull cows (Gonzalez et al., 2008), which further exemplifies that muscles of beef cattle have differential responses to RAC.

Muscle type is a major factor in determining the rate of meat discoloration (Hood, 1980). The fiber type composition and biochemical processes with which the fibers of the muscle utilizes, affects the visual color of the meat. Faustman and Cassens (1990) reported that the fiber type composition of steaks under aerobic display affects the rate of discoloration through accumulation of metmyoglobin by altering the rate of oxygen diffusion and consumption, autoxidation of myoglobin in the presence of oxygen, and rate of metmyoglobin reducing activity (**MRA**). Of these factors, McKenna et al. (2005) and Bekhit and Faustman (2005) identified MRA as the basis for meat discoloration by reducing metmyoglobin accumulation.

The MRA of muscle is strongly affected by the fiber composition of muscle due to the type of metabolism utilized within the fiber. Type I fibers rely more heavily on oxidative metabolism than type II fibers, which utilize glycolytic metabolism (Aberle et al., 2001). Because of these differences in metabolism, type I fibers contain more mitochondria than type II fibers. Giddings (1974) hypothesized and others confirmed (Arihara et al., 1996) that mitochondria contribute to metmyoglobin reduction by supplying NADH as a reducing cofactor. Kim et al. (2008) confirmed muscle fiber type has an effect on NADH presence by demonstrating that porcine muscle with a high percentage of oxidative fibers contains more NADH. Based on these findings and the fiber type shift toward glycolytic in the current study, one would expect RAC treated muscles to exhibit less MRA. The fiber type percentage shift needed to affect the MRA

of muscles remains unknown, but the current data suggests that it must be larger than 21%. Steaks which exhibited muscle fiber type shifts due to RAC supplementation did not exhibit the expected changes in MRA as measured by NOM reducing ability.

The inability to detect changes in metmyoglobin reduction may be related to the assay of choice as the role of the MRA system and tests used to measure it remain controversial (Bekhit and Faustman, 2005). Beef MRA can be measured by total reducing activity, aerobic reducing ability, metmyoglobin reductase activity, and NOM reducing assays (Sammel et al., 2002; McKenna et al., 2005). Previous reports indicate that NOM is intermediately correlated (r = 0.61) with metmyoglobin accumulation (McKenna et al., 2005) and only correlated well with metmyoglobin accumulation when discoloration differences were the largest (Sammel et al., 2002). However, Ledward (1972) reported that aerobic reducing ability is highly correlated (r = -0.94) to metmyoglobin concentration, and Sammel et al. (2002) found this assay to be the best method for measuring reducing ability. Therefore, employment of additional methods to measure MRA than the one utilized in this study may help explain the surface discoloration data reported on day 5 of the study and resolve the discontinuity found between the NOM reducing data and the fiber type shift.

Conclusion

Supplementing beef steers with 200 $mg \bullet hd^{-1} \bullet d^{-1}$ of RAC for the final 28 days of feeding induced the MyHC fiber shift in all muscles observed except the SM. The shift observed was not consistent between muscles, which confirm that RAC differentially affects muscles in young beef cattle. The expected effects of the RAC induced shift on NOM reducing ability, L*a*b* values, and $(K/S)_{572}/(K/S)_{525}$ ratio was not seen. Therefore, a greater shift toward type II fibers may be required to affect these shelf-life characteristics. However, visual panel surface discoloration scores indicated that by day five of the study RAC had a detrimental effect on color

stability. The inconsistencies between the MRA results and visual panel surface discoloration may be due to the test used to measure MRA, but requires further research.

Table 5-1. Least squares means of muscle fiber myosin heavy chain isoform distribution, cross-sectional area and diameter from six muscles from steers fed with and without ractopamine-HCl

	Type I Fiber		Type II Fiber	
Muscle	Percentage	Area (μm^2)	Percentage	Area (μm^2)
Adductor				
Control	32.7[a]	1,644.3	67.3[a]	2,957.0
Ractopamine	30.7[b]	1,774.0	69.3[b]	2,865.6
Gracilis				
Control	37.9[a]	1,312.1	62.1[a]	1,786.3
Ractopamine	33.6[b]	1,396.1	66.4[b]	1,727.7
Longissimus lumborum				
Control	32.5[a]	2,244.6	67.5[a]	3,333.8
Ractopamine	30.5[b]	2,323.6	69.5[b]	3,318.2
Rectus femoris				
Control	13.8[a]	1,179.0	86.3[a]	2,032.3
Ractopamine	15.8[b]	1,356.5	84.2[b]	2,245.1
Semimembranosus				
Control	27.9	1,893.8	72.2	3,267.0
Ractopamine	27.5	2,001.6	72.5	3,616.6
Vastus lateralis				
Control	22.4[a]	1,704.5	77.6[a]	2,581.2
Ractopamine	17.6[b]	1,523.8	82.4[b]	2,369.1
SEM		187.0		187.0

[a,b] Means within a muscle and column with different letters are significantly different ($P < 0.05$).

Table 5-2. Percent nitric oxide metmyoglobin reduced and ratios of oxy- and metmyoglobin accumulation from steaks originating from six muscles of steers fed with and without ractopamine-HCl displayed under simulated retail display conditions for 5 days

Muscle		*Adductor*		*Gracilis*		*Longissimus lumborum*		*Rectus femoris*		*Semimembranosus*		*Vastus lateralis*	
Item[1]	Day	CON	RAC	CON	RAC	CON	RAC	CON	RAC	CON	RAC	CON	RAC
NOM Reduced[2]	0	66.8[a]	81.7[b]	62.8	61.8	70.6	75.6	28.8	32.7	82.0	79.9	61.5	68.2
	1	44.0[a]	62.5[b]	54.7	47.3	84.4	76.4	28.3	20.6	63.0	64.9	57.7	62.3
	2	36.7	38.7	48.6	49.4	57.9[a]	74.9[b]	20.3	14.6	49.6	56.8	52.0	59.8
	3	31.1	27.8	49.3	48.2	61.5	71.8	20.4	15.9	43.2	34.3	50.4	47.0
	4	15.3	18.4	40.2	39.7	50.7	62.4	19.5	14.3	31.1	26.5	50.0	52.6
	5	15.4	11.3	23.8	26.5	44.9	36.8	24.8	18.2	28.6	24.6	40.7	43.4
	SEM	5.5		5.5		5.5		5.5		5.5		5.5	
$(K/S)_{572}/(K/S)_{525}$[3]	0	1.27	1.28	1.34	1.28	1.33	1.34	1.34	1.29	1.33	1.30	1.34	1.35
	1	1.13	1.14	1.31	1.31	1.36	1.34	1.21	1.20	1.24	1.26	1.29	1.34
	2	1.06	1.05	1.27	1.22	1.30	1.28	1.13	1.13	1.13	1.16	1.30	1.24
	3	0.99	0.97	1.23	1.22	1.27	1.26	1.08	1.10	1.12	1.11	1.21	1.17
	4	0.95	0.92	1.19	1.20	1.28	1.27	1.04	1.04	1.10	1.01	1.15	1.14
	5	0.90	0.84	1.06	1.11	1.23	1.24	1.01	0.98	1.06	0.99	1.14	1.07
	SEM	0.03		0.03		0.03		0.03		0.03		0.03	
$(K/S)_{610}/(K/S)_{525}$[4]	0	0.17	0.17	0.21	0.22	0.16	0.17	0.18	0.20	0.16	0.17	0.17	0.17
	1	0.20	0.22	0.18	0.19	0.12	0.14	0.25	0.21	0.15	0.15	0.13	0.14
	2	0.23	0.25	0.19	0.19	0.14	0.14	0.21	0.22	0.15	0.15	0.15	0.17
	3	0.27	0.28	0.20	0.21	0.14	0.14	0.22	0.23	0.14	0.17	0.16	0.18
	4	0.29	0.30	0.22	0.22	0.14	0.15	0.22	0.25	0.17	0.18	0.16	0.19
	5	0.28	0.31	0.26	0.26	0.13	0.14	0.23	0.25	0.18	0.19	0.16	0.19
	SEM	0.02		0.02		0.02		0.02		0.02		0.02	

[a-b]Means within a row without common superscript significantly differ ($P < 0.05$).

[1]CON = steers supplemented 0 mg•hd^{-1}•d^{-1} of ractopamine; RAC = steers supplemented 200 mg•hd^{-1}•d^{-1} of ractopamine.

[2]Percent nitric oxide metmyoglobin reduced.

[3] Ratios derived from Kubelka-Munk equations calculated from spectrophotometer measurements taken at 572 nm and 525 nm used to estimate the percentage of Metmyoglobin accumulation. Smaller values indicate an increase in Metmyoglobin.

[4] Ratios derived from Kubelka-Munk equations calculated from spectrophotometer measurements taken at 610 nm and 525 nm used to estimate the percentage of Oxymyoglobin accumulation. Larger values indicate a decrease in Oxymyoglobin.

Table 5-3. Least squares means of HunterLab MiniScan XE L*, a*, and b* values from steaks originating from six muscles of steers fed with and without ractopamine-HCl displayed under simulated retail display conditions for 5 days

Muscle		*Adductor*		*Gracilis*		*Longissimus lumborum*		*Rectus femoris*		*Semimembranosus*		*Vastus lateralis*	
Item[1]	Day	CON	RAC	CON	RAC	CON	RAC	CON	RAC	CON	RAC	CON	RAC
L*[2]	0	49.1	49.3	45.7	44.9	43.6	41.9	47.9	47.7	43.5	42.7	44.4	44.5
	1	46.3	46.1	44.9	43.5	42.4	42.3	45.7	46.9	42.8	40.8	42.1	41.8
	2	45.4	45.2	44.3	43.3	42.5	41.0	43.8	44.4	42.0	41.5	41.7	41.8
	3	45.5	44.7	44.4	42.2	41.4	40.1	43.7	44.4	41.5	40.4	41.2	41.8
	4	43.4	44.2	44.1	42.7	40.8	39.7	42.5	42.4	41.3	40.1	41.1	40.8
	5	44.9	43.5	43.5	41.8	40.3	39.2	43.6	43.6	41.3	40.0	41.0	41.0
	SEM	1.6		1.6		1.6		1.6		1.6		1.6	
a*[3]	0	32.8	32.3	30.7	29.7	33.2	33.3	32.4	30.4	34.7	34.0	34.7	33.7
	1	28.3	27.1	30.8	32.2	36.5	35.2	30.9	28.6	33.6	34.4	35.9	35.6
	2	24.9	24.2	30.0	30.0	34.2	34.6	28.6	26.7	32.0	31.7	33.4	31.4
	3	22.7	22.1	28.3	28.8	33.9	34.5	27.1	25.1	30.9	30.4	32.2	29.3
	4	22.3	21.1	27.3	27.6	33.8	33.8	25.9	25.0	29.9	29.1	31.4	29.4
	5	20.8	19.5	23.6	24.4	33.3	32.9	24.8	22.8	28.5	27.6	30.0	27.7
	SEM	1.4		1.4				1.4		1.4		1.4	
b*[4]	0	30.6	30.1	26.4	25.5	30.4	30.4	29.2	27.3	32.5	31.9	31.5	30.5
	1	28.6	27.6	27.0	28.5	34.2	32.8	29.5	27.2	32.2	33.3	33.6	33.1
	2	26.5	26.2	26.2	26.6	31.9	32.1	28.2	26.3	31.3	31.1	31.5	29.7
	3	25.6	25.5	25.4	26.0	31.9	32.1	27.3	25.5	30.6	30.7	30.8	28.4
	4	25.5	24.9	24.7	24.8	31.7	31.7	27.1	25.7	29.8	29.8	30.1	28.5
	5	25.0	24.6	23.1	23.4	31.4	31.0	25.9	24.6	29.1	29.0	29.3	27.6
	SEM	1.1		1.1		1.1		1.1		1.1		1.1	

[1]CON = steers supplemented 0 mg•hd^{-1}•d^{-1} of ractopamine; RAC = steers supplemented 200 mg•hd^{-1}•d^{-1} of ractopamine.
[2] Lightness: 100 = White; 0 = Black.
[3] Redness: 60 = Red; -60 = Green.
[4] Blueness: 60 = Yellow; -60 = Blue.

Table 5-4. Least squares means of visual panel scores for steaks from six muscles displayed under simulated retail display conditions for 5 days from cattle fed with and without ractopamine-HCl

Muscle		*Adductor*		*Gracilis*		*Longissimus lumborum*		*Rectus femoris*		*Semimembranosus*		*Vastus lateralis*	
Item[1]	Day	CON	RAC	CON	RAC	CON	RAC	CON	RAC	CON	RAC	CON	RAC
Beef Lean Color[2]	0	5.5	5.4	5.7	5.9	5.5	5.3	5.8	5.6	5.4	5.4	5.6	5.3
	1	4.5	4.3	5.3	4.9	5.4	5.3	5.7	5.2	5.0	4.7	5.1	5.0
	2	4.1	3.9	5.1	4.9	5.4	5.2	5.6	5.4	5.0	4.6	4.9	4.9
	3	3.9	3.7	4.9	4.4	5.3	4.9	5.6	5.3	4.7	4.4	5.0	4.6
	4	3.6	3.3	4.4	4.2	5.0	4.9	5.3	5.3	4.5	4.2	4.9	4.7
	5	3.4	3.2	4.3	4.3	5.1	4.7	5.5	5.3	4.5	4.1	5.0	4.5
	SEM	0.3		0.3		0.3		0.3		0.3		0.3	
Fat Color[3]	0	1.9	1.9	2.0	2.0	2.0	2.0	1.8	1.8	1.9	1.9	1.9	1.8
	1	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.1	2.0	2.0
	2	2.0	2.1	2.2	2.1	2.1	2.1	2.0	2.1	2.1	2.1	2.1	2.0
	3	2.0	2.0	2.1	2.1	2.0	2.0	2.1	2.0	2.0	2.1	2.0	2.0
	4	2.4	2.5	2.4	2.3	2.3	2.3	2.2	2.2	2.2	2.2	2.2	2.2
	5	2.7	2.6	2.6	2.7	2.4	2.4	2.3	2.4	2.3	2.4	2.2	2.4
	SEM	0.1		0.1		0.1		0.1		0.1		0.1	
Surface Discolor[4]	0	1.2	1.1	1.0	1.0	1.1	1.0	1.1	1.1	1.0	1.1	1.1	1.0
	1	2.5	2.6	1.1	1.1	1.0	1.0	1.7	1.8	1.3	1.5	1.3	1.4
	2	3.8^{a}	4.3^{b}	1.2	1.0	1.1	1.0	2.0	2.2	1.7	1.9	1.6	1.8
	3	4.5	4.8	1.3	1.5	1.0	1.1	2.5	2.7	2.1	2.4	2.1	2.5
	4	4.9	5.1	1.6	1.9	1.3	1.3	2.8	3.0	2.5	2.8	2.5^{a}	3.0^{b}
	5	5.1	5.3	2.5	2.8	1.6	1.6	2.9^{a}	3.6^{b}	2.7^{a}	3.2^{b}	3.0^{x}	3.4^{y}
	SEM	0.2		0.2		0.2		0.2		0.2		0.2	

[a-b]Means within a row without common superscript significantly differ ($P < 0.05$).

[x-y]Means within a row without common superscript tend to differ ($P < 0.10$).

[1] CON = steers supplemented 0 mg•hd^{-1}•d^{-1} of ractopamine; RAC = steers supplemented 200 mg•hd^{-1}•d^{-1} of ractopamine.

[2]8 = Extremely bright cherry-red; 7 = Bright cherry-red; 6 = Moderately bright cherry-red; 5 = Slightly bright cherry-red; 4 = Slightly dark cherry-red; 3 = Moderately dark red; 2 = Dark red; 1 = Extremely dark red.

[3]5 = Yellow; 4 = Moderately yellow; 3 = Slightly yellow; 2 = Creamy white; 1 = White.

[4]7 = Total discoloration (100%); 6 = Extensive discoloration (80-99%); 5 = Moderate discoloration (60-78%); 4 = Modest discoloration (40-59%); 3 = Small discoloration (20-39%); 2 = Slight discoloration (1-19%); 1 = No discoloration (0%).

CHAPTER 6
EFFECT OF RACTOPAMINE-HCl ON LIVE AND CARCASS CHARACTERISTICS WHEN FED TO STEERS DURING THE FINAL 28 DAYS OF FEEDING

Introduction

The supplement ractopamine-HCl (**RAC**) belongs to a class of compounds called beta-adrenergic agonists (**BAA**). The Food and Drug Administration approves the use of RAC in both swine (Paylean® in 1999) and cattle (Optaflexx® in 2003). Ractopamine is approved for cattle fed in confinement during the last 24 to 42 days of feeding before slaughter. Ractopamine supplementation during the final days of feeding commonly improves live performance by increasing average daily gain and gain to feed ratio in steers (Gruber et al., 2007) and heifers (Quinn et al., 2008). In steers, RAC supplementation increased gain to feed ratio and ADG by 17.2% and 15.3%, respectively (Gruber et al., 2007). Schroeder et al. (2005a) reported that RAC supplementation can improve average daily gain and gain to feed ratio by as much as 26%.

Through its repartitioning ability, ractopamine elevates skeletal muscle protein deposition at the expense of fat deposition. Numerous studies report that ractopamine increases hot carcass weight and ribeye area, decreases fat, and increases dressing percentage by as much as 3.6% (Schroeder et al., 2005b; Winterholler et al., 2007). While the majority of published data on ractopamine documents its effects on whole carcass parameters, little data exists on yields from muscles throughout the carcass of beef cattle. In pigs, RAC increases wholesale cut yields, trimmed wholesale cut yields, and boneless, wholesale cut yields (Crome et al., 1996). Stites et al. (1991) found that RAC increased trimmed loin and ham yields, but did not affect the yields of the boston butt and picnic shoulder. Plascencia et al. (1999) reported that the BAA, zilpaterol-HCl, increased the percentages of the knuckle, skirt, neck, and inside round in cattle. In addition, RAC supplemented to cull cows differentially increased muscle fiber hypertrophy of various

muscles originating from the chuck and round (Gonzalez et al., 2008). Therefore, RAC also may have the ability to increase yields from individual muscles of supplemented carcasses.

In today's market condition with of high corn/feed prices, the use of RAC to improve average daily gain, gain to feed ratio, and carcass characteristics becomes an attractive option for producers to lower the cost of beef production. The objective of the study was to investigate the effect of RAC on live performance and carcass characteristics, while also evaluating its effect on whole muscle yields from the carcass.

Materials and Methods

Animals and Pre-Harvest Diets

This experiment was approved by the University of Florida Institutional Animal Care and Use Committee. Thirty-four crossbred steers housed at the University of Florida Beef Teaching Unit were selected for the study. Steers were separated into four harvest groups (three groups of eight and one group of ten) and followed the same implantation program consisting of a Ralgro implant (36 mg Zeranol) followed by a Revalor-S implant (120 mg trenbolone acetate and 24 mg estradiol). Once a harvest group was established, steers were separated into two pens so that breed type, initial pen weight, and visual backfat thickness were similar. This separation occurred approximately two weeks before the beginning of each RAC supplementation period to allow the steers time to establish a new pen dynamic.

At 6 p.m. daily, steers were fed a basal concentrate diet (Table 1) in concrete bunks that provided 76.2 cm per head of bunk space. To allow the steers time to adjust to the top dress, both the control and treatment pens were supplemented with a hand mixed blank top dress (0 $mg \bullet hd^{-1} \bullet d^{-1}$ of RAC; Lakeland Animal Nutrition, Lakeland, FL) at a rate of 0.45 kg per head per day for two weeks before the beginning of the 28 day RAC supplementation period. Once the supplementation period began, the control pen continued to receive the hand mixed blank top

dress at a rate of 0.91 kg per head per day. The treatment pens received 0.91 kg per head per day of hand mixed top dress designed to provide 200 mg•hd^{-1}•d^{-1} of RAC (Elanco Animal Health, Greenfield, IN) for 28 days prior to harvest.

Harvesting and Carcass Data Collection

Following day 28 of supplementation, final steer body weights (**BW**) were collected and steers were transported to the University of Florida Meats Laboratory for harvesting. Steers were harvested under Federal Inspection following the Humane Methods of Slaughter Act of 1978. No carcasses from any harvest group were electrically stimulated during the harvesting process. During harvest, weights of inedible offal items were collected, including head, feet, pluck, empty rumen, and hide weights. Following harvest, hot carcass weights (**HCW**) were recorded and carcasses were placed in a cooler at 0°C. Approximately thirty-six hours postmortem, carcasses were ribbed at the 12^{th}-13^{th} rib juncture and allowed to bloom for 30 minutes prior to carcass data collection. An experienced university employee collected carcass data including lean and bone maturity, marbling score, color score, texture score, firmness score, 12^{th} rib fat, 12^{th} rib ribeye area, kidney heart and pelvic fat (**KPH**), and average preliminary yield grade. These measurements were used to calculate dressing percent, USDA Yield grade and Quality grade (USDA, 1997).

Whole Muscle Extraction and Measurement

Seventy-two hours postmortem, subprimals including the bone-in strip loin, knuckle, and top round were excised from the right side of each carcass. From the subprimals, whole muscles were removed. The *Longissimus lumborum* (**LL**) was removed from the bone-in strip loin, and the *Adductor* (**ADD**), *Gracilis* (**GRA**), and *Semimembranosus* (**SM**) were removed from the top round. Similarly, the *Rectus femoris* (**RF**) and *Vastus lateralis* (**VL**) were removed from the knuckle.

After separation, each muscle was trimmed to 0.635 cm of fat and the commodity weight was taken. Muscles were then trimmed of all visible fat and epimysium connective tissue, and reweighed for a denuded weight measurement. Finally, muscle length, width, maximum depth, and minimum depth measurements were taken on each muscle with a measuring tape. Prior to vacuum packaging and aging, muscle L*a*b* values were measured using a HunterLab MiniScan XE (HunterLab, Reston, VA). Whole muscles were placed in heat shrink vacuum bags (B2570; Cryovac, Duncan, SC), vacuum packaged using a Multivac C500 (Multivac, Inc., Kansas City, MO), and wet aged for 13 days postmortem at 2 ± 3°C.

Warner-Bratzler Shear Force Analysis

After aging, muscles were removed from their vacuum bags, weighed for purge loss, and cut into six 1.27 cm steaks and one 2.54 cm steak. Steaks were cut from the same end of each muscle, perpendicular to the orientation of the muscle fibers. The first six steaks were used for shelf-life analysis (data presented elsewhere). The seventh 2.54 cm steak was vacuum packaged and stored at -40°C for Warner-Bratzler shear force analysis. Twenty-four hours prior to cooking, steaks were thawed at 4 ± 2°C. Steaks were cooked on preheated Hamilton Beach Indoor/Outdoor open top grills (Hamilton Beach Brands, Washington, NC) following the guidelines of the American Meat Science Association. Thermocouples (Omega Engineering, Inc., Stamford, CT) were placed in the geometric center of each steak and internal temperatures were constantly monitored and recorded using 1100 Labtech Notebook for Windows 1998 (Computer Boards, Inc., Middleboro, MA). Steaks were cooked to an internal temperature of 71°C, turned once at 35°C (AMSA, 1995). Cooked steaks were chilled at 4 ± 2°C for 24 hours. Once chilled, six 1.27 cm cores were obtained from each steak, parallel to the orientation of the muscle fibers. Each core was sheared once through the center of the core and perpendicular to

the orientation of the muscle fiber using an Instron Universal Testing Machine (Instron Corporation, Canton, MA) with a Warner-Bratzler head (crosshead speed of 200 mm/min).

Statistics

Live performance and carcass data was analyzed as a randomized complete block design with harvest group as the blocking factor and treatment as the fixed effect. Pen was considered the experimental unit for live performance and carcass data. Muscle and Warner-Bratzler shear force data were analyzed as a split-plot design with repeated measures. Kill group and treatment was considered the whole plot and muscle was considered the sub-plot. Kill group × treatment was utilized as the random error for the whole-plot, and kill group × the treatment/muscle interaction was considered the random error for the sub-plot. All measured variables were analyzed with the PROC MIXED procedure of SAS (SAS Inst. Inc., Carry, NC, 2002). Pair-wise comparisons between the least square means of the factor levels were computed using the PDIFF option of the LSMEANS statement. Differences were considered significant at an alpha = 0.05 and tendencies at an alpha = 0.15.

Results

Live performance for both treatment groups was recorded during the final 28 days of feeding (Table 6-2). At the beginning of the 28 day supplementation period, both treatment groups' body weights were not significantly different ($P = 0.55$). During the supplementation period, both treatment groups had similar ($P > 0.05$) dry matter intake, ADG, and G:F ratio. At the end of the trial period, body weights for both treatment groups were not significantly different ($P = 0.70$) due to both groups having similar ($P = 0.34$) gains during the final 28 days of feeding.

During the harvesting process, inedible offal weights were collected, and 36 hours later, postmortem carcass data was collected (Table 6-3). The weights and percentage of total body

weight of the head, pluck, viscera, feet, and hide were not significantly ($P > 0.05$) affected by the inclusion of RAC in the diet. Important carcass measurements that indicate increased muscling, including hot carcass weight, longissimus muscle area, and longissimus muscle area per 100 pounds of HCW were unaffected ($P > 0.05$) by RAC supplementation. However, RAC supplementation tended ($P = 0.15$) to increase dressing percentage. Carcass lean quality parameters including color and texture scores were unaffected ($P > 0.05$) by RAC supplementation. Lean maturity of RAC supplemented animals appeared ($P = 0.02$) older, and RAC also tended ($P < 0.15$) to soften the firmness of the lean of supplemented animals. Marbling score was significantly ($P < 0.0001$) decreased by RAC supplementation. However, quality grade was unaffected ($P = 0.64$) by RAC supplementation. Other carcass fat measurements, namely 12th rib fat thickness and KPH were not affected ($P > 0.05$) by RAC supplementation. Therefore, this resulted in both treatment groups having similar ($P = 0.94$) yield grades.

Commodity weights, denuded weights, muscle dimensions, and Warner-Bratzler shear force values are presented in Table 6-4. Muscle weights and dimensions were measured with the hope of demonstrating the ability of RAC to improve muscling of steers. However, RAC did not significantly ($P > 0.05$) affect the muscle weights or dimensions of most of the muscles analyzed. Ractopamine did significantly ($P = 0.02$) increase the width of the GRA and tended ($P = 0.08$) to increase the width of the LL. Ractopamine supplementation also tended ($P = 0.12$) to increase the minimum depth of the GRA.

The effect of RAC on whole muscle color and tenderness was also analyzed (Table 6-4). For all six muscles observed except the RF, RAC supplementation did not affect ($P > 0.05$) L*a*b* values. Ractopamine supplementation tended ($P < 0.11$) to cause a darker appearance

in the RF. Warner-Bratzler shear force analysis indicated that the tenderness of steaks from all six muscles were unaffected by RAC supplementation ($P > 0.05$).

Discussion

Since RAC was approved for use in beef cattle in 2003, numerous studies report that the inclusion of RAC in the diet during the final days of feeding prior to harvest improves both live performance and carcass characteristics. Therefore, producers have incentive to utilize this feed additive in their feeding program as a means to lower costs of gain. Commonly, ADG and G:F ratio are the live performance characteristics improved by supplementation. However, in the current study, all live performance characteristics measured were unaffected by RAC supplementation over the final 28 days of feeding. Winterholler et al. (2007) reported that the same feeding protocol increased ADG and G:F ratio by 4.6 and 3.8%, respectively. In addition, Winterholler et al. (2007) found that RAC supplementation increased DMI by 3.5%, which contrasts the findings of this study and most other published studies. Schroeder et al. (2005a) and Laudert et al. (2005a) both found that RAC supplemented at 200 $mg \bullet hd^{-1} \bullet d^{-1}$ increased both ADG and G:F ratio by as much as 20%. In steers of differing biological type, RAC increased ADG and G:F ratio without having a biological type interaction (Gruber et al., 2007). This finding suggests that the lack of RAC effect in the current study was not due to the utilization of steers with differing genetic backgrounds.

The numerical live performance values from this study were similar to significant values published in other studies. In the current study ADG and G:F ratio were increased numerically by 10 and 7%, respectively. These values are in the range of significant values published by Wineterholler et al. (2007) and Gruber et al. (2007). Overall, gain data is the most encouraging data from the live performance data. Published studies indicate that RAC can increase gain significantly by 6% (Winterholler et al., 2007). In the current study, RAC increased gain 9%

over the final 28 days of feeding. The glaring difference between the present study and published studies is the large difference in number of animals used. The lack of significance found in this study may be due to the low number of animals used and the variation in individual animal response when compared to other studies.

Insignificant live performance data also may be due to the RAC dosage level and duration employed. We used the regimen suggested by the manufacturer of 200 mg•hd^{-1}•d^{-1} for the final 28 days of feeding. In the registration trials used to gain FDA approval, RAC supplemented at 100, 200, and 300 mg•hd^{-1}•d^{-1} improved ADG linearly by 17.1, 19.6, and 25.7%, respectively (Schroeder et al., 2004). Abney et al. (2007) investigated the effects of supplementing 100 and 200 mg•hd^{-1}•d^{-1} of RAC for 28, 35, and 42 days. These researchers found that RAC supplementation at 100 mg•hd^{-1}•d^{-1} yielded a linear increase in growth performance characteristics as time increased, with a maximum benefit at day 42. However, RAC supplemented at 200 mg•hd^{-1}•d^{-1} produced a quadratic increase in the same parameters and maximum benefit was reached at day 35. The researchers concluded that RAC fed at 200 mg•hd^{-1}•d^{-1} for 35 days provided optimal live performance. These findings suggest that modifying our regimen to include RAC supplementation at 200 mg•hd^{-1}•d^{-1} for 35 days or 100 mg•hd^{-1}•d^{-1} for 42 days may yield a greater live performance response.

Ractopamine supplementation produces mixed carcass characteristic results. Effects commonly include modest increases in HCW, dressing percentage, and ribeye area in steers, with no changes in 12th rib fat thickness or other carcass characteristics (Schroeder et al., 2004; Schroeder et al., 2005b; Laudert et al., 2005b). Walker et al. (2006) reported that RAC increased HCW by 6.9 kg, and tended to increase ribeye area by 1.74 cm in heifers. Gruber et al. (2007) demonstrated that RAC administration to cattle differing in biological type increased HCW and

ribeye area by 2 and 4%, respectively. In the current study, RAC supplementation did not increase HCW, longissimus muscle area, or longissimus muscle area per 100 pounds of HCW. In agreement, Quinn et al. (2008) reported that a variety of RAC supplementation regimens, including the protocol utilized in this study, had no effect on any carcass characteristics. Numerical increases in ribeye area in the current study are similar to the significant increases in recent published studies, indicating that the lack of significance could be a result of the lower number of animals used.

When feeding RAC to cattle, producers are often concerned about is its effect on fat deposition. In the current study, RAC supplementation did not affect 12th rib fat thickness and KPH, which contributed to both treatment groups having similar yield grades. Schroeder et al. (2004) reported that RAC supplemented at 200 mg•hd^{-1}•d^{-1} did not alter 12th rib fat thickness or KPH, but tended to decrease the yield grade of steers. In contrast, Sissom et al. (2007) found that RAC supplementation at the same dosage and time interval decreased 12th rib fat thickness and yield grades. Winterholler et al. (2008) found that when 200 mg•hd^{-1}•d^{-1} of RAC was supplemented for 37 days 12th rib fat thickness was unaffected, but yield grade was decreased. However, when the supplementation period was reduced to 28 days, there was no effect on 12th rib fat thickness or yield grade. In general, most studies report that RAC supplemented at 200 mg•hd^{-1}•d^{-1} does not affect 12th rib fat thickness or yield grade (Laudert et al., 2005b; Walker et al., 2006; Abney et al., 2007; Quinn et al., 2008). In the current study marbling score was decreased by supplementation. However, concern about this effect should be minimal since RAC supplemented steers achieved in the same 'Slight' category of marbling as the non supplemented steers and quality grade was unaffected. Gruber et al. (2007) also found that RAC supplementation tended to lower marbling, but not enough to affect quality grade. Therefore, the

literature and the current study indicate that RAC supplemented at 200 mg•hd^{-1}•d^{-1} for 28 days prior to harvest has little to no detrimental effect on fat measurements or yield and quality grades.

Ractopamine supplementation tended to improve dressing percentage. Since RAC supplementation did not affect the weights or percentage of total body weight of the head, pluck, empty rumen, feet, or hide, differences between treatment groups' inedible offal item weights did not contribute to this improvement. Early published studies from Schroeder et al. (2005b) and Laudert et al. (2005b) reported that RAC supplementation improves dressing percentage. However, recent studies published by Gruber et al. (2007), Quinn et al. (2008), and Winterholler et al. (2007, 2008) found that RAC supplementation was unable to increase dressing percentage. The increase in dressing percentage without large decreases in fat measurements may indicate that RAC supplementation increased muscling.

To date, RAC-induced increases in ribeye area is the only indicator of increased muscling. As discussed earlier, this result is mixed across studies and is not pronounced. However, commodity weights, denuded weights, and muscle dimensions of the six muscles analyzed in the current study suggest that RAC supplemented at 200 mg•hd^{-1}•d^{-1} for the final 28 days of feeding has no ability to globally increase muscling. In agreement, Dijkhuis (2007) reported that RAC administered in a similar manner did not affect the same measurements of the same six muscles from cull cows. Fiber morphometric data from the LL and SM of these cull cows found that RAC supplemented at 200 mg•hd^{-1}•d^{-1} did not increase muscle fiber hypertrophy in type I or IIA fibers of the LL, and only increased type IIA fibers by 34% in the SM. The 34% increase in type IIA fibers of the SM was not sufficient to cause an overall increase in muscle weight or dimensions (Gonzalez et al., 2008). In the current study, fiber morphometric data (Gonzalez,

Chapter 5) indicated that RAC did not stimulate hypertrophy in either muscle fiber isoform from any of the six muscles examined. Thus, the inability of RAC supplementation to stimulate muscle fiber hypertrophy results in a lack of whole muscle growth.

Reduced muscle protein degradation plays an active role in muscle protein accretion due to BAA supplementation (Koohmaraie et al., 1991). Specifically, BAAs may increase activity of the postmortem proteolytic enzyme inhibitor, calpastatin, by as much as 348% (Koohmaraie et al., 1992, Garssen et al., 1995). While not officially identified as the main culprit of decreased meat tenderness, increased calpastatin activity may contribute to decreases in tenderness. When RAC is supplemented at high levels, such as 300 mg•hd^{-1}•d^{-1}, steak tenderness is decreased (Schroeder et al., 2004; Avendano-Reyes et al., 2006). At a lower dosage level (200 mg•hd^{-1}•d^{-1}), Quinn et al. (2008) reported that RAC supplementation did not affect steak tenderness. However, RAC supplemented at 200 mg•hd^{-1}•d^{-1} for 28 days decreased the tenderness of steaks from cattle of three different biological backgrounds when measured by Warner-Bratzler shear force and trained sensory panel (Gruber et al., 2008).

All of these studies examined the tenderness of steaks originating from the longissimus dorsi in steers. However, the effect of RAC supplementation on other muscles of the carcass is unknown. In cull cows, RAC supplemented at 200 mg•hd^{-1}•d^{-1} did not affect tenderness in the same six muscles when compared to controls (Dijkhuis et al., 2008). In the current study, RAC supplemented at the same dosage level also did not affect the tenderness, as measured by Warner-Bratzler shear force, of any of the muscles observed. The ADD, GRA, LL, and RF were all similar in tenderness. The SM and VL were similar in tenderness to one another, but were less tender than the other muscles. However, steaks from all muscles except the VL, regardless

of treatment group, were considered acceptably/intermediately tender (Miller et al., 1995; Miller et al., 2001) when analyzed by Warner-Bratzler shear force.

Quinn et al. (2008) stated that since neither HCW nor ribeye area were increased by RAC supplementation, muscling was not increased by supplementation and it would be expected for RAC to yield no effect on meat tenderness. In addition, Gruber et al. (2008) hypothesized that because RAC is a β_1-agonist, decreases in tenderness were due to increased protein synthesis and muscle fiber hypertrophy. However, RAC did not increase the cross-sectional area or diameter of the muscle fibers in any of the muscles examined (Gonzalez, Chapter 5). Therefore, the lack of a RAC effect on muscle tenderness in the current study is not surprising, given that RAC supplementation did not affect measurements of muscling or muscle fiber hypertrophy.

Conclusion

Data indicates that feeding RAC at 200 $mg \bullet hd^{-1} \bullet d^{-1}$ for the final 28 days before slaughter has little or no effect on live, carcass, and individual muscle characteristics. This study provides valuable data documenting the effects of 200 $mg \bullet hd^{-1} \bullet d^{-1}$ of RAC on muscle dimension, weights, and tenderness of numerous muscles of the beef carcass. While ADG, G:F ratio, and ribeye area numerical differences were comparable to significant published values, more animals may be needed to detect significant differences. Employing a feeding strategy (greater RAC dosage or feeding time) different than the one followed in the current study may be necessary to elicit more beneficial effects on both live performance and carcass characteristics.

Table 6-1. Composition of basal diet[1] and carrier for top dress[2]

Item	Amount, percent of DM
Basal Diet	
Whole corn	85.0
Cottonseed hulls	7.5
Protein pellet	7.5
Trace minerals	0.05
Carrier	
Dehydrated alfalfa meal	50.8
Corn meal	33.3
Calcium carbonate[3]	12.5
Soybean oil	1.7
Mineral oil	1.7

[1] Diet provided 75.1% TDN, 11.9% crude protein, 3.3% crude fat, 6.5% crude fiber, 9.9% acid detergent fiber, 16.1% neutral detergent fiber, 0.17% calcium, and 0.28% phosphorus.

[2]Carrier provided 91.4% DM, 11.8% crude protein, 5.6% crude fat, 16.9% crude fiber, and 62.1% TDN.

[3]Calcium carbonate was reduced to 7.5% when ractopamine-HCl was added to the carrier. All nutrient values remained the same.

Table 6-2. Least squares means of live performance characteristics of steers supplemented with and without ractopamine-HCl[1]

Item	Control	Ractopamine, 200 mg•hd^{-1}•d^{-1}	SEM
Initial BW, kg	531.58	522.78	10.32
Final BW, kg	564.32	558.45	10.73
DMI, kg/d/pen	39.14	39.69	2.48
Gain, kg	32.68	35.62	2.30
ADG, kg	1.20	1.31	0.09
G:F	0.13	0.14	0.01

[1]Live performance characteristics measured during the final 28 days of feeding.

Table 6-3. Least squares means of offal weights and carcass characteristics of steers supplemented with and without ractopamine-HCl

Item	Control	Ractopamine, 200 mg•hd^{-1}•d^{-1}	SEM
Offal Weights			
Head, kg	13.98	14.15	0.44
Head Percentage[1]	2.49	2.53	0.06
Feet, kg	10.34	10.38	0.26
Feet Percentage[1]	1.84	1.86	0.04
Pluck, kg	7.59	7.42	0.26
Pluck Percentage[1]	1.35	1.33	0.04
Empty Rumen, kg	56.27	55.05	2.72
Empty Rumen Percentage[1]	9.94	9.85	0.43
Hide, kg	41.81	41.84	1.58
Hide Percentage[1]	7.42	7.52	0.28
Carcass Performance			
HCW, kg	344.09	345.45	7.57
Dressing Percent[2]	60.92^{x}	61.86^{y}	0.98
Lean Maturity[3]	145.29^{a}	156.47^{b}	3.96
Bone Maturity[4]	150.79	148.44	5.93
Marbling Score[5]	335.88^{a}	324.12^{b}	4.40
Color Score[6]	3.20	3.48	0.25
Texture Score[7]	3.36	3.53	0.23
Firmness Score[8]	2.06^{x}	2.47^{y}	0.20
12th rib fat, cm	0.92	0.93	0.14
LM area, cm^2	85.04	87.36	3.29
LM/100 kg HCW[9]	24.78	25.38	0.71
KPH	2.17	2.28	0.10
Yield Grade	2.61	2.60	0.19
Quality Grade[10]	16.35	16.11	0.35

[a-b]Means within a row without common superscript significantly differ ($P < 0.05$).
[x-y]Means within a row without common superscript tend to differ ($P < 0.15$).
[1]Percentage of live weight.
[2]Dressing Percentage = (HCW/ Final BW)* 100.
[3]100 = A; 200 = B; 300 = C; 400 = D; 500 = E.
[4]100 = A; 200 = B; 300 = C; 400 = D; 500 = E.
[5]100 = Practically Devoid; 200 = Traces; 300 = Slight; 400 = Small.
[6]1 = Bright Cherry Red; 8 = Extremely Dark Red.
[7]1 = Very Fine; 7 = Extremely Course.
[8]1 = Very Firm; 7 = Extremely Soft.
[9]Longissimus muscle area per 100 kg of hot carcass weight.
[10]13-15 = Standard; 16-18 = Select; 19-21 = Choice; 22-24 = Prime.

Table 6-4. Least squares means of muscle weights, dimensions, color measurements, and Warner-Bratzler shear force values from six muscles of the round from cattle fed with and without ractopamine-HCl

	Adductor		*Gracilis*		*Longissimus lumborum*		*Rectus femoris*		*Semimembranosus*		*Vastus lateralis*		SEM
Item[1]	CON	RAC	CON	RAC	CON	RAC	CON	RAC	CON	RAC	CON	RAC	
Commodity, kg	1.76	1.67	2.15	2.26	5.93	5.79	1.78	1.80	5.55	5.53	2.02	2.11	0.19
Percent HCW[2]	0.51	0.49	0.62	0.65	1.73	1.68	0.52	0.52	1.61	1.60	0.59	0.61	0.04
Denuded, kg	1.57	1.50	1.06	1.07	3.94	4.12	1.60	1.61	4.98	4.97	1.71	1.80	0.12
Percent HCW[2]	0.46	0.44	0.31	0.31	1.14	1.19	0.47	0.47	1.44	1.43	0.50	0.52	0.02
Length, cm	21.26	20.82	32.20	31.34	39.89	39.31	23.71	25.27	34.08	34.42	25.56	26.09	0.79
Width, cm	14.85	14.50	18.13[a]	19.54[b]	16.02[x]	17.10[y]	13.06	12.94	19.95	19.28	18.39	19.07	0.45
Minimum Depth, cm	2.13	1.98	1.27[x]	1.57[y]	3.04	3.12	2.21	2.43	2.02	2.16	1.89	1.81	0.43
Maximum Depth, cm	9.18	9.02	2.95	3.00	6.26	6.41	8.40	8.35	11.65	11.78	7.45	7.37	0.19
L*	33.61	33.32	33.50	34.03	37.98	36.72	50.79[x]	48.30[y]	36.77	35.39	41.69	40.25	1.08
a*	26.41	26.25	22.82	23.64	27.43	28.22	27.98	27.58	27.70	27.48	28.56	28.49	2.80
b*	18.22	18.75	12.73	13.96	20.45	20.98	21.83	20.46	20.09	19.52	20.91	20.58	4.73
WBS[3], kg	3.25	3.50	3.78	3.31	3.49	3.21	3.65	3.52	4.14	3.84	5.11	4.67	0.25

[a-b]Means within a row without common superscript significantly differ ($P < 0.05$).

[x-y]Means within a row without common superscript tend to differ ($P < 0.15$).

[1] CON = steers supplemented 0 mg•hd^{-1}•d^{-1} of ractopamine; RAC = steers supplemented 200 mg•hd^{-1}•d^{-1} of ractopamine.

[2]Muscle weight percentage of hot carcass weight.

[3]Warner-Bratzler shear force values.

CHAPTER 7
OVERALL CONCLUSIONS AND IMPLICATIONS

Supplementing cull cows with 200 mg•hd^{-1}•d^{-1} of RAC for the final 28 days of a 92 day feeding period increased *Longissimus dorsi* type I muscle fiber CSA by 31% when compared to controls. Addition of an implant to RAC fed cull cows increased type I fiber CSA by an additional 13% when compared to non-implanted RAC supplemented cows. Neither RAC supplementation nor employment of an implant strategy affected the CSA of type IIA muscle fibers in the *Longissimus dorsi*. When RAC was combined with an implant, muscle fiber isoform distribution altered, with type I fibers shifting to type IIA fibers. Ractopamine supplementation did not affect the number of satellite cells or fiber associated nuclei counted when identified with immunohistochemical techniques. The lack of an effect on both fiber associated nuclei and satellite cell numbers indicates that the hypertrophy of type I muscle fibers stimulated by RAC supplementation could be due to alteration in protein synthesis/degradation rates. Because older animals have a limited ability to synthesize protein, it is hypothesized that this caused the limited increases in muscle fiber hypertrophy experienced when feeding RAC to younger animals.

Various concentration levels (100, 200, and 300 mg•hd^{-1}•d^{-1}) of RAC supplemented to cull cows during the final 28 days of feeding differentially affected the morphometrics of various muscles throughout the carcass. Increasing the dosage level of RAC in the diet did not linearly increase the CSA of type I or type IIA fibers of muscles originating from the chuck, loin, or round. The inclusion of RAC in the diet at any level did not affect the CSA of type I fibers in the *Longissimus dorsi* as was seen when RAC was supplemented for 35 days. When RAC supplementation affected muscle fiber CSA, improvements in CSA were minimal. Interestingly,

the lower concentration of RAC only increased fiber size in type I and II muscle fibers of the INF, a muscle characterized by a higher proportion of red fibers.

Ractopamine addition at any of the three supplementation levels caused a biological effect on muscle fiber isoform distribution. Muscle fibers of the *Longissimus dorsi*, *Semimembranosus*, and *Vastus lateralis* demonstrated shifts from type I to IIA, while fibers of the *Infraspinatus* showed shifts from type IIA to I. Since no cows were implanted in this trial, this indicates that an implant is not needed to cause a shift in myosin heavy chain isoform distribution. Ractopamine supplementation at all levels decreased the number of fiber associated nuclei counted in multiple muscles. This provides evidence that fiber associated nuclei incorporation into the muscle fiber is not needed for RAC induced muscle fiber hypertrophy. This finding further substantiates the hypothesis that RAC stimulated hypertrophy is most likely accomplished by altering protein synthesis/degradation rates.

When RAC was supplemented to steers at 200 $mg \bullet hd^{-1} \bullet d^{-1}$ during the final 28 days of feeding, results indicated that RAC has little to no effect on live, carcass, or individual muscle characteristics. While RAC did not significantly improve live performance, including average daily gain and gain to feed ratio, numerical differences were similar to significant published values. Ribeye area also demonstrated numerical increases in cross-sectional area with RAC supplementation similar to published values. It is hypothesized that using more animals in the study could have added significance to these values.

The literature has failed to define the effects of RAC supplementation on other muscles of the beef carcass. When RAC was supplemented to steers at 200 $mg \bullet hd^{-1} \bullet d^{-1}$ for the final 28 days before slaughter, muscle dimensions, weights, and tenderness of muscles of the loin and round were unaffected. The lack of increased muscling can be attributed to the inability of RAC to

increase the CSA of either type I and IIA muscle fibers in all muscles evaluated. However, RAC did stimulate the muscle fiber isoform shift seen in cull cows. The shift in isoforms was not consistent among all the muscles evaluated; further indicating that RAC has a differential effect on muscles.

While RAC supplementation did not affect metmyoglobin reducing ability or objective measures of color stability during retail display, trained panelists did find that RAC supplemented steaks had a higher percentage of surface discoloration toward the end of the five day display period. This could indicate the RAC supplementation detrimentally affects meat color stability, but this warrants further research. If RAC affects shelf-life through a reduction in metmyoglobin reducing ability, further research using different tests to measure metmyoglobin reducing ability are needed.

The literature published over the last four years concerning the supplementation of RAC to beef cattle reveals two trends. Firstly, in the live animal, RAC supplementation improves average daily gain and gain to feed ratio with little to no effect on dry matter intake. Second, RAC supplementation modestly increases ribeye area, can occasionally decrease yield grade, and rarely affects carcass fat measurements. While RAC does increase ribeye area, it seems that its ability to increase muscling is limited. The goal of this dissertation was to describe the cellular events responsible for the lack of a gross increase in muscling in multiple muscles of old and young beef cattle. Results indicate that RAC supplementation was unable to significantly stimulate muscle fiber hypertrophy in type I and type IIA muscle fibers in muscles originating from the chuck, loin, or round. Immunohistochemical analysis indicated that RAC supplementation did not increase the numbers of satellite cells or fiber associated nuclei. Therefore, RAC may stimulate muscle hypertrophy by altering protein synthesis/degradation

rates. Because older animals have a limited capacity to synthesize protein, it was hypothesized that RAC may not be able to stimulate muscle fiber hypertrophy in cull cows. However, results that RAC also has a limited ability to stimulate hypertrophy of muscle fibers from young steers, which resulted in an inability to increase muscle weights or dimensions. Therefore, the results from cull cows may not be due to an inability of RAC to stimulate increases in synthesized protein, but possibly the inability of RAC to decrease protein degradation.

In the literature, researchers report that most BAAs stimulate muscle hypertrophy by altering protein degradation rates through increased calpastatin activity. As a consequence of this increased activity, the studies report decreases in tenderness. However, in the current body of work, the tenderness of various muscles was unaffected by RAC supplementation. The lack of growth observed may be attributed to a failure of RAC to stimulate increased calpastatin activity and reduce protein degradation in the muscle fiber. Recently, the FDA approved the use of a new BAA in beef cattle, labeled as a β_2-receptor agonist. Results from a few studies indicate that this product has a greater capacity to increase muscling. Steaks from cattle fed this product become less tender, which may indicate that growth is occurring through increased calpastatin activity. Muscles of beef cattle contain more β_2-receptors than β_1-receptors. Ractopamine is labeled as a β_1-agonist. Lower levels of β_1-receptors in the muscles of beef cattle may limit the ability of RAC to increase calpastatin activity, which lowers protein degradation and consequently yields greater muscle mass. To date, the effect of RAC on protein degradation rates, specifically its effect on calpastatin activity, has not been examined and requires further research.

Producers must consider what type of benefits they hope to reap from employing RAC supplementation in their feeding program. While published data and the current body of work

indicate that RAC can positively affect live performance, the effects at the carcass level are minimal. Therefore, producers must decide if the improvement in the live performance of their beef cattle outweighs the cost of feeding the supplement. They also must be aware that there will be little benefit to the carcass from feeding RAC due to its inability to stimulate muscle fiber hypertrophy. The beef industry must consider that whole muscle cuts from RAC supplemented cattle can have decreased shelf-life due to shifts in muscle fiber isoforms, and evaluate if the decrease in shelf-life is worth the potential losses at retail. Finally, because RAC has a limited ability to increase muscling in beef carcass, researchers and producers may consider feeding RAC at different dosages or increase the length of the supplementation period to elicit greater effects.

APPENDIX A
REPRESENTATIVE PHOTOMICROGRAPHS OF *INFRASPINATUS* MUSCLE IMMUNOSTAINED FOR DETECTION OF SATELLITE CELLS

Methodology for staining can be found in the immunohistochemistry section of the materials and methods section of chapters 3 and 4. Satellite cells were identified as Hoechst dye and α-Pax7 positive nuclei located outside the α-dystrophin barrier.

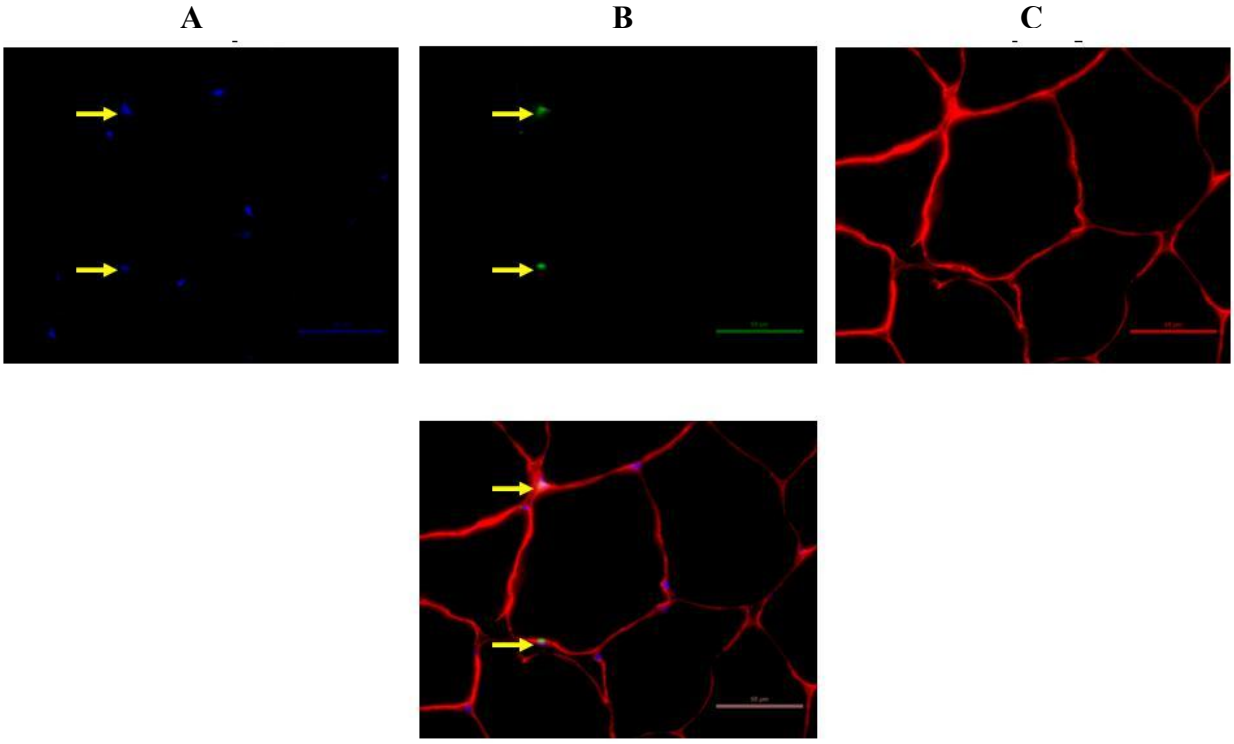

Figure A-1. Photomicrographs of immunohistochemical stains used to identify satellite cells. A) Hoechst Dye; B) α-Pax7; C) α-Dystrophin; D) Combined stain photomicrographs.

APPENDIX B
REPRESENTATIVE PHOTOMICROGRAPH OF *LONGISSIMUS DORSI* MUSCLE IMMUNOSTAINED FOR DETECTION OF FIBER ASSOCIATED NUCLEI

Methodology for staining can be found in the immunohistochemistry section of the materials and methods section of Chapters 3 and 4. Fiber associated nuclei were identified as propidium iodide stained objects within the α-dystrophin barrier.

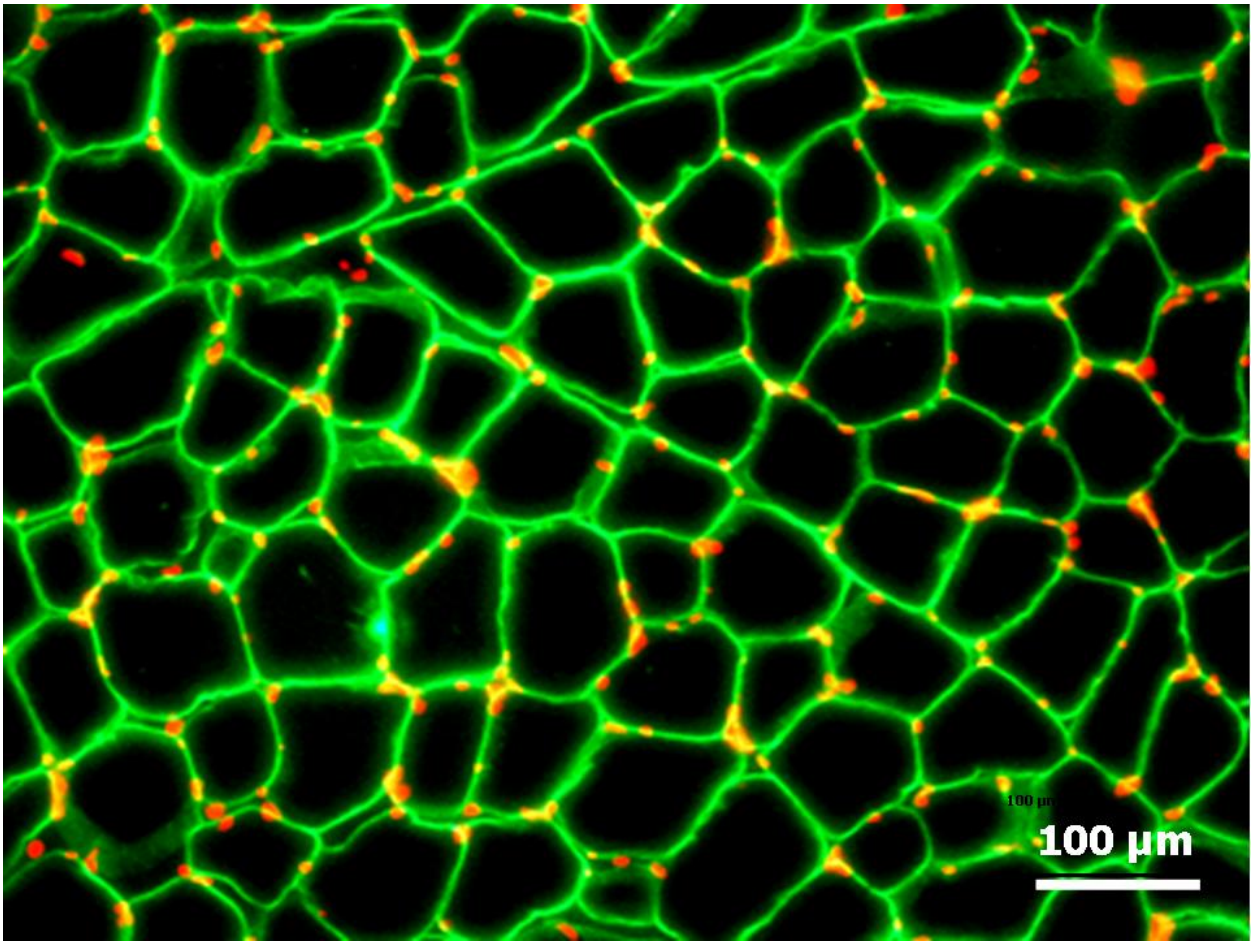

Figure B-1. Photomicrograph of *Longissimus dorsi* muscle immunostained for fiber associated nuclei detection. α-Dystrophin identified as green stain and propidium iodide stain (red stain) identified nuclei.

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BIOGRAPHICAL SKETCH

John Michael Gonzalez was born in San Antonio, Texas in 1978 to Joe Luis Gonzalez and Linda Martha Gonzalez. He was the youngest of three children born in the Gonzalez family. His older sister, Rebecca Lynn Gonzales is a school teacher in San Antonio, Texas; and his older brother Joe Luis Gonzalez, Jr. is a loan officer living in Austin, Texas. John Michael began his formal education attending Charles C. Ball Elementary School from grades kindergarten through five. John Michael was then given the great opportunity to attend Saint Mary's Hall School where he attended both middle and high school. While attending Saint Mary's Hall, John Michael was exposed to agriculture by visiting ranches belonging to his friends' families and by helping his best friend rope steers.

Upon graduation from Saint Mary's Hall, John Michael attended Texas A&M University in College Station, Texas. When deciding which major to purse in college, John Michael decided that he enjoyed all aspects of production agriculture and decided to major in agricultural economics (with an emphasis in farm and ranch management); and also to major in poultry science. While attending A&M, John Michael received his first agriculture-related employment opportunity when he was hired for a job in the Texas A&M University Department of Veterinary Physiology and Pharmacology, managing a herd of miniature Sinclair swine. At this time, John Michael swore to his boss that he would never pursue any form of graduate education. Toward the end of his education at Texas A&M, John Michael gained experience managing a 10,000 acre cow-calf cattle operation located in Enicnal, Texas. At the Triple-Bar ranch, John Michael assisted in the daily operations associated with maintaining the cow-calf herd, while also managing the nutrition program for the ranch's trophy whitetail deer operation. During this experience, John Michael realized that he would have to win the mega-lottery to have an operation of this size and scope and would need to make other life plans.

Upon graduation from Texas A&M in May of 2002, John Michael quickly broke his promise to his boss at the Texas A&M Veterinary School and decided to pursue his Master's degree at Sul Ross State University in Alpine, Texas. While attending this little school located in the most beautiful country of Texas, John Michael decided to pursue his Master's in meat science under Paul A. Will. Because of the large population of meat goats located in West Texas, John Michael decided to study the effects of preharvest supplementation methods on goat meat quality. John Michael's thesis was titled *The Effects of Vitamin D_3 on Goat Meat Tenderness and Color Stability*. In between his studies and research, John Michael spent time helping the West Cattle Company and working at a restaurant at the Hotel Paisano in Marfa, Texas. At Sul Ross, John Michael coordinated and taught all the General Animal Science classes, and also assisted in all meat science related courses. For his efforts, John Michael was awarded Outstanding Graduate Student in Animal Sciences and Graduate Student Teaching Award of Merit by the North American Colleges and Teachers of Agriculture and Sul Ross State University in 2003. After many trial and tribulations (and no meat lab), John Michael received his Master of Science degree in May of 2002.

Upon completion of his Master's degree, John Michael moved to Gainesville, Florida to work on his Doctorate degree in meat science under the supervision of D. Dwain Johnson in the Department of Animal Sciences at the University of Florida. For his Doctorate program, John Michael worked in collaboration with Sally Johnson, examining the effects of ractopamine supplementation on the muscle biology of beef animals. During his time at the University of Florida, John Michael extensively aided the Department of Animal Sciences to fulfill its mission of teaching and extension by serving as a graduate assistant in several undergraduate courses and state extension programs. As a testament to his hard work and dedication during his four years at

the University of Florida, John Michael has published two peer reviewed articles and received the 2008 Animal Science Graduate Student Association Ph.D. Student of the Year Award. Upon receiving his doctorate, John Michael hopes to enter the NASA Astronaut Candidate Program to conduct research for the United States space program. John Michael is also considering a position in the meats industry or academia. His main goal in life is to have a career that is adventurous, exciting, that will make him and his family happy.