SEQUENTIAL FEEDING OF β-ADRENERGIC AGONISTS TO REALIMENTATED CULL COWS

by

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B.S., University of Tennessee at Martin, 2006
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AN ABSTRACT OF A DISSERTATION

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Abstract

Sixty cull cows were utilized to investigate the effects of feeding a single or sequence of β-adrenergic agonists (β-AA) on performance, mRNA expression, carcass traits, economics, meat palatability, and ground beef color. Treatments included: 1) concentrate fed for 74 d (**C**); 2) concentrate fed for 49 d then supplemented with ractopamine-HCl for 25 d (**RH**); 3) concentrate fed for 51 d then supplemented with zilpaterol-HCl for 20 d (**ZH**); 4), concentrate fed for 26 d then supplemented with RH for 25 d followed by ZH for 20 d (**RH + ZH**). No differences existed among treatments for performance or carcass characteristics. However, cows supplemented with ZH (ZH and RH + ZH treatments) had increased LM areas ($P = 0.18$) compared to control and RH cows. Sequential feeding of RH followed by ZH had no influence on β_2-adrenergic receptor (AR) mRNA expression. However, β_2-AR mRNA was increased ($P < 0.05$) in the RH and ZH treatments when RH or ZH was supplemented during the last 20 to 25 d of feeding. Myosin heavy chain (MHC) Type IIa mRNA decreased ($P < 0.05$) from d 24 to 51 in all cows, while MHC-IIx increased ($P < 0.05$) in the ZH and RH + ZH treatments during ZH supplementation. No differences were observed in ground beef color shelf-life among treatments. Effects of β-AA supplementation on meat palatability varied among muscles. *Infraspinatus* steaks had improved ($P < 0.05$) WBSF values with β-AA supplementation. *Psoas major* steaks from the RH + ZH treatment were rated as more tender than steaks from all other treatments. Non-enhanced LM steaks from ZH supplemented cows had higher ($P = 0.12$) WBSF values along with decreased ($P < 0.0001$) percentages of degraded desmin compared to control and RH cows. Collagen solubility of the LM was increased with ZH supplementation compared to RH and control cows. Enhancement of steaks with 0.1 M calcium lactate improved LM tenderness of β-AA supplemented cows. Implanting and feeding cull cows for 74 d, regardless of β-AA supplementation, added value by transiting cows from a "cull" cow to "white" cow market.

Key Words: realimentation, cull cows, ractopamine-HCl, zilpaterol-HCl, carcass and meat traits, β_2-adrenergic receptors

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Dedication

I would like to dedicate this dissertation to my husband, Casey Weber. I could not have reached this success without his love and encouragement.   His continual patience and support do not go unappreciated.

Preface

This manuscript is written according to the style guidelines of ***the Journal of Animal Science***, a scientific journal encompassing the many facets of animal science research. **Chapter 1** is a general introduction, including the overall research objectives and statement of hypothesis. **Chapter 2** is a review of the literature pertaining to cull cow meat, realimentation programs, growth promoting agents, and postmortem considerations in regards to cow meat. **Chapter 3** is an investigation into the effects of sequential feeding of β-adrenergic agonists on growth performance, carcass characteristics, cellular responses, and economics of production in mature cow realimentation programs. **Chapter 4** is a continuation of the project presented in Chapter 3, but discusses effects related to muscle characteristics and postmortem enhancement technologies. **Chapter 5** draws general conclusions regarding sequential feeding of β-adrenergic agonists and utilization of growth promoting agents in cull cow realimentation.

Chapter 1 - General Introduction

Cows are often culled from herds because of problems associated with old age, reproductive inefficiency, disposition, lameness, or unsatisfactory performance. Selling cull cows can account for 15 to 20% of the income associated with cow-calf operations (Yager et al., 1980). Unlike young steers and heifers that have been finished in a feedlot, a majority of cows harvested are from live-auction markets. Therefore, much variation exists in the quality and composition of cow carcasses (Woerner, 2010). In addition, culling decisions are often made at weaning in the fall of the year when cows have poor body condition due to nutritional demands of lactation and deterioration in forage quality. With a majority of cows being culled in September and October, cow prices are typically lowest in fall months when the market is saturated (Little et al., 2002). Considering this seasonal effect, it can be beneficial to feed cull cows through the winter to take advantage of higher prices in the Spring, while also improving the value of cow carcasses (Feuz, 1999).

Researchers have found that feeding cows a high-energy ration prior to harvest can lead to increased HCW, improved muscling, brighter lean color, whiter fat color, increased collagen solubility, and improved tenderness (Wooten et al., 1979; Miller et al., 1987; Boleman et al., 1996; Schnell et al., 1997; Sawyer et al., 2004). In a recent study by Woerner (2009), aggressively feeding cows a high-energy ration for 95 d led to superior improvements in HCW, LM area, marbling score, intramuscular fat percentage, lean color, fat color, and tenderness compared to non-fed beef cows. Additionally, supplementing cows with a high-energy ration prior to harvest can improve meat sensory profiles. Stelzleni and Johnson (2010) reported that fed cull beef cows had a lower occurrence of off-flavors than non-fed beef cows.

Feeding a concentrate ration alone can improve market value and carcass characteristics of cull cows; however, the use of anabolic steroid implants and/or β-adrenergic agonists (β-AA) have been explored as options to more efficiently improve carcass yields and value of mature cows. Implanting cows in combination with a high plane of nutrition can improve live weight gain, HCW, carcass muscling, LM area, and meat yield in mature cows (Cranwell et al., 1996b; Funston et al., 2003). In contrast,

the use of β-AA, such as ractopamine-HCl (RH) and zilpaterol-HCl (ZH), may provide minimal benefits in mature cow feeding systems. Allen et al. (2009) found no effect of RH on growth performance or carcass traits in cull dairy cows. Although numerical improvements in HCW and dressing percentage were observed in RH-fed cows, minimal statistical advantages were found when comparing RH supplemented rations to high-energy rations without β-AA supplementation (Holmer et al., 2009a). Neill et al. (2009) found that implanting and supplementing cull cows with ZH increased LM areas, but no significant improvements were observed in any additional performance or carcass traits compared to just implanting and feeding a high-energy ration.

Ractopamine-HCl is classified as β_1-AA and preferentially binds to β_1-adrenergic receptors (AR). Zilpaterol-HCl is classified as a β_2-AA and primarily binds to β_2-AR (Moody et al., 2000). There was a trend for RH supplementation during the last 28 d of finishing to increase β_2-AR mRNA levels in heifers (Sissom et al., 2007) and steers (Winterholler et al., 2007). Gonzalez et al. (2008) reported similar findings in mature cows indicating that there was a trend for β_2-AR mRNA levels in the *semimembranosus* to be increased after feeding RH for 28 d.

I hypothesized, based on published β_1 and β_2-AA research, that feeding RH for 25 d followed by ZH for 20 d would lead to additive or synergistic effects due to RH promoting an up-regulation of β_2-AR. Therefore, the objectives of this research were to determine the effects of feeding a single or sequence of β-AA to mature beef cows on growth performance, carcass composition and quality, LM mRNA levels throughout feeding, economics of production, muscle biochemical characteristics, and meat sensory traits.

Chapter 2 - Review of Literature

CULL COW MARKET AND MEAT CHARACTERISTICS

Classification of market cows

Cows culled from cow-calf, seedstock, or dairy operations account for 17 to 19% of all cattle harvested in the U.S. each year equaling 6-million head and approximately 80,000 metric tons of boneless beef (Woerner, 2010). Most cows are procured by packing plants through auction barns based on body condition scores. Cows that have body condition scores greater than 6, on a 1 to 9 scale, have greater live value (Apple, 1999). Cows with body condition scores of 7, 8, or 9 are sold as "breakers" and their carcasses may be fabricated into boneless, whole-muscle cuts (Hodgson et al., 1992). Cows with body condition scores of 4, 5, or 6 are sold as "boners" and meat from these cows is often utilized for some merchandisable cuts and the remainder for ground products (Hilton et al., 1998). The "Lean" and "Light" carcass classifications are used for cows with a body condition score less than 4, and meat from these carcasses is used almost solely for ground or comminuted products (Peel and Doye, 2008). Cull cows that are younger than 42 mo of age (A and B maturity carcasses) can be classified using the USDA quality grades of Utility, Standard, Select, Choice, and Prime. However, most cull cows are greater than 42 mo of age and are only eligible for USDA grades of Commercial, Utility, Cutter and Canner. Even though cow carcass grades exist, they are rarely used in industry because they do not reflect common trade practices, and the expense of grading is not cost-effective (Wise, 1994). Instead, most packers sort carcasses based on fat color, lean color, amount of muscling, and degree of marbling (Woerner, 2010). Carcasses from cows that have been fed a high-energy ration prior to harvest are easily separated from non-fed carcasses because they have increased muscling and whiter external fat. "White-fat" cows almost always yield a premium price because packers sell middle meats and other whole-muscle cuts from these carcasses to foodservice companies (Woerner, 2010).

The most recent audit (NCBA, 2007) suggested that cows and bulls are sold in better condition than in 1999 evidenced by beef cows with heavier HCW, increased

muscling, and more desirable fat color scores.   Additionally, the audit revealed that an increased percentage of plants were fabricating subprimals from cow carcasses (Figure 2.1). All mature cow and bull facilities included in the audit were fabricating a portion of carcasses into merchandisable ribeye and tenderloin cuts. This demonstrates that, in the current market, beef from mature cow carcasses is being utilized for food service cuts in addition to ground beef and sausage production.
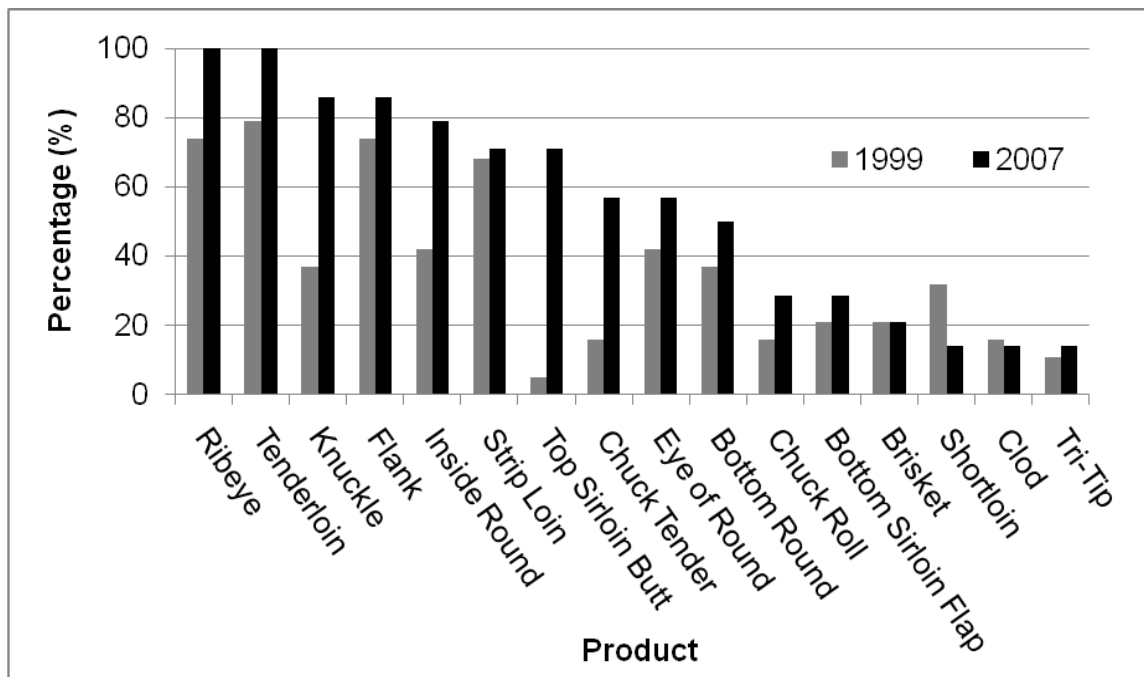
Figure 2.1 Percentage of plants that produce selected merchandisable cuts (*Adapted from National Market Cow and Bull Beef Quality Audit; NCBA, 2007*).

Meat from mature, non-fed cows

Although notable improvements have been made during the past decade, numerous problems still are associated with carcasses from cows. A recent study found that non-fed beef cows had lower HCW, smaller LM areas, less fat thickness (FT), inferior muscling, yellower external fat color, and darker lean color than A-maturity, Select grade steer carcasses (Stelzleni et al., 2007). Additionally, when beef from mature cows is compared to grain-finished steers, beef is found to be tougher, less juicy, and to have more undesirable off-flavors (Woerner, 2010).

It is well documented that carcass maturity and tenderness are inversely related. Tuma et al. (1962) found that Warner Bratzler shear force (WBSF) measurements and sensory panel evaluations indicated decreased tenderness with advancing animal age, but that greater differences in tenderness were noted between 18 and 42 mo than between 42 and 90 mo of age. This agrees with early research (Dunsing, 1959; Simone et al., 1959) who concluded that the optimal age for tenderness may fall between 18 to 20 mo of age. Breidenstein et al. (1968) and Berry et al. (1974) noted that, when compared to A and B maturity, E maturity carcasses have decreased tenderness, as observed by trained sensory panel ratings and WBSF measurements. Smith et al. (1982) reported that WBSF values of *longissimus, semimembranosus, biceps femoris, and semitendinosus* muscles increased as carcass maturity increased. More recently, Stelzleni et al. (2007) found that WBSF values of 8 muscles from non-fed beef cows were higher than WBSF values of muscles from A-maturity, Select grade beef steers. The only muscle not differing in WBSF values between non-fed cows and Select grade steers was the *psoas major*.

Several mechanisms can be responsible for the decreased tenderness of beef from mature cows. First, as animals mature, collagen becomes more cross-linked and thus more heat stable. This results in less collagen becoming solubilized during cooking. Increased collagen cross-linking can be correlated with decreased meat tenderness (Goll et al., 1964; Hill, 1966; Herring et al., 1967; Light et al., 1985). The percentage of soluble collagen in steaks from 22-mo-old steers was reported to be 8.5% while the percentage of soluble collagen in 7-year-old cows was found to be significantly lower at only 1.8% (Hill et al., 1966). Secondly, non-fed cow carcasses are often leaner than young steer and heifer carcasses and may chill faster causing cold toughening. Increased subcutaneous fat was found to improve tenderness by allowing carcasses to chill more slowly and to increase enzyme activity (Smith et al., 1976). Lastly, postmortem proteolysis may be slower in mature cow carcasses. Huff-Lonergan et al. (1995) reported that muscle samples from cows had slower rates of postmortem degradation than samples from younger steers.

Flavor profiles from non-fed beef cows can also contribute to negative characteristics of cow meat. Stelzleni and Johnson (2010) observed that sensory

panelists rated off-flavor in non-fed cow steaks as more intense than off-flavors in steaks from A-maturity, Select grade steers. The most common descriptor used to characterize off-flavors associated with non-fed cow meat was "grassy". The "grassy" off-flavor that consumers find unappealing in meat from mature beef is likely caused by non-fed cows consuming a forage-based diet rather than a high-energy concentrate ration. Hilton et al. (1998) determined that as fat color transitions from white to yellow, the incidence of off-flavors increased. This is because forage based diets are high in beta-carotene, which is the primary pigment responsible for yellow fat color development in beef (Dunne et al., 2008).

Negative attributes associated with beef from mature carcasses should be addressed so that cull cows can satisfy a need for intermediate priced middle meats and further processed products. Realimentation programs coupled with utilization of growth promoting agents could be beneficial in increasing value of mature cow carcasses.

FEEDING CULL COWS PRIOR TO HARVEST

Growth and performance

Numerous researchers have investigated realimentation feeding programs that vary in diet composition and number of days on feed. The most highly utilized and most effective feeding strategy is to supplement cows with a concentrate ration for 50 to 100 d prior to harvest. Supplementing cull cows with a high-energy ration will take advantage of a cow's potential for compensatory gain. Swingle et al. (1979) reported that cows fed an 80% concentrate ration consumed less feed while having higher carcass gains than moderately fed (40% concentrate ration) cows. In agreement with this research, Miller et al. (1987) found that cows supplemented with a high-energy ration for 84 d had higher ADG than cows fed a maintenance ration for 84 d.

Cow performance is often lowest during the earliest and latest stages of finishing. Matulis et al. (1987) reported that from 0 to 28 and 57 to 84 d of feeding cows had lower ADG than during the 29 to 56 d range. Research by Schnell et al. (1997) noted that

ADGs were negative the first 2 wk of feeding but increased from d 14 to 28 before leveling off. Cows, regardless of ration energy level, showed negative G:F during d 0 to 14, highest G:F from d 15 to 42, and an intermediate G:F from d 43 to 54 (Sawyer et al., 2004). This fluctuation in cow performance occurs because cows are becoming acclimated to rations during the first few weeks of supplementation. Then, in the later stages of feeding, compensatory gain dissipates.   Feeding cows a high-energy ration for at least 50 d can efficiently improve cow weights. Supplementation for extended periods may further enhance carcass characteristics and sensory properties, but efficiency of gain may be decreased.

Carcass characteristics

In a recent study conducted by Stelzleni et al. (2007), carcass data surveyed from commercial populations of cattle indicated that fed beef cows had superior carcass characteristics compared to non-fed beef cows. Fed cows had increased HCW, LM muscle area, marbling, FT, KPH, and yield grade along with improvements in, lean maturity, bone maturity, overall maturity, fat color, and lean color compared to non-fed cows. Swingle et al. (1979) reported that supplementation of cows with a high-energy ration (80% concentrate) compared to a moderate-energy ration (40% concentrate) for approximately 57 d improved carcass gain, but the composition of gain did not differ. Additionally, Wooten et al. (1979) observed that feeding cows, regardless of low versus moderate energy level, increased HCW, dressing percentage (DP), FT, LM area, and marbling score along with the moderate-energy ration increasing the percentage of shortloin. When comparing high-energy versus low-energy rations, Miller et al. (1987) concluded that feeding high-energy rations for 84 d increased final quality grade by improving marbling score, lean maturity, and overall maturity while also increasing USDA yield grade due to increased FT and KPH.

The exact number of days required to cause carcass improvements is varied in published literature. In an early study evaluating the impact of days on feed, Matulis et al. (1987) reported that within 28 d of feeding a high-energy ration, improvements were observed in HCW, LM area, marbling, and quality grade. However, cows fed for extended periods (56 and 84 d) demonstrated even greater improvements in HCW, LM

area, lean maturity, and quality grade. Boleman et al. (1996) observed that increasing the number of days on feed generally led to a linear increase in live weight, HCW, and FT. However, after 28 d on feed, lean color and texture reached maximum improvement. After 56 d on feed LM area and fat color reached maximum improvement. To see maximum improvement in marbling score, 84 d on feed was required. Yield grade was significantly increased from 56 to 84 d on feed, but the yield grade after 84 d was still considered acceptable.

In summary, most research indicates that carcass characteristics and lean meat yields can be improved sufficiently after 28 to 56 d on feed. However, longer feeding periods may prove beneficial in improving tenderness and other sensory properties.

Muscle and sensory characteristics

Muscle and sensory characteristics such as fat color, lean color, tenderness, and sensory profiles can be improved by cull cow realimentation. Stelzleni et al. (2007) reported that steaks from fed cows had superior lean and fat color compared to non-fed cows. Fat color improves with supplementation because less β-carotene from grass is present in fat deposits (Dunne et al., 2008). French et al. (2000) suggested that whitening of fat is due to a dilution effect of new fat being deposited that has less carotene in it. Typically, sufficient improvements in fat color are seen after 28 to 56 d of supplementation with a high-energy ration (Boleman et al., 1996; Cranwell et al., 1996b; Schnell et al., 1997).

Miller et al. (1987) found after 84 d on a high-energy ration, cows had improved lean color. Cranwell et al. (1996b) found that L* values were improved after 28 d of feeding, but that 56 d of supplementation was required to see improvements in a* values, hue angle, saturation index, and subjective lean color scores. In general, the amount of myoglobin present in muscle increases with animal age, thus leading to darker red beef color (Bowling et al., 1977). Additionally, the environment of pasture versus feedlot finished cattle may impact lean color. Cattle finished on pasture have increased physical activity resulting in darker beef color. Plus, feedlot cattle are exposed to more human interaction thus decreasing stress during transportation and

harvest, which may offset the risk of high carcass pH (Bowling, 1977). Nonetheless, even though the exact mechanism is not understood, data suggests that supplementing cows with a high-energy ration for at least 28 d can lead to brighter lean color.

Tenderness and sensory profiles can also be improved by feeding a high-energy ration prior to harvest. Miller et al. (1987) reported that 10-yr old cows supplemented with a high-energy ration for 84 d had decreased WBSF values along with improved sensory scores for fragmentation and connective tissue amount. Boleman et al. (1996) demonstrated that as days on feed increased, tenderness was improved. These researchers went on to quantify differences in collagen and found that the percentage of soluble collagen was increased when cows were fed for 56 d compared with only being fed 28 d. Cranwell et al. (1996a) reported that LM steaks from cows supplemented for 28 to 56 d had improved sensory ratings for myofibrillar tenderness, connective tissue amount, and overall tenderness, as well as decreased WBSF values. Schnell et al. (1997) observed that feeding cows for 56 d did not improve WBSF values, but that LM steaks were rated by panelist as more tender than steaks from non-fed cows. Cranwell et al., (1996a) and Schnell et al. (1997) both reported that the amount of soluble collagen tended to increase as days on feed increased, but reached a plateau after 28 d. As animals age, increased collagen cross-linking occurs. However, feeding cows increases the amount of newly synthesized collagen creating more heat-liable cross-links (Aberle et al., 1981).

Conflicting results are reported on the influence of supplementation on flavor profiles and the number of days on feed required to observe differences in flavor attributes. Even though values for off-flavor intensity were relatively low (2.4 and 2.1 on a 1 = no off flavor and 8 = extremely intense off-flavor scale), Miller et al. (1987) reported that feeding a high-energy ration increased off-flavor intensity compared to feeding a low-energy ration. Schnell et al. (1997) reported no differences in flavor intensity or flavor attributes in *bicep femoris* or *longissimus* steaks from cows fed a high-energy ration for 14, 28, 42, or 56 d. In contrast to theses studies, Faulkner et al. (1989) reported that fed-cows had improved beef flavor intensity and overall desirability after 42 d on feed, but no further improvements were observed by increasing the number of days on feed to 84. Furthermore, their data showed no difference in off-

flavor intensity between steaks from cows fed for 0, 42 and 84 d. Cranwell et al.,
(1996b) also reported that flavor intensity scores increased by feeding cattle for at least
28 d, but that further improvements were not observed at 56 d. Boleman et al. (1996)
found that off-flavor intensity was decreased after 28 d of feeding and beef flavor
intensity was increased after 56 d on feed. More recently, Stelzeleni et al. (2008)
characterized off-flavors in cows fed for 0, 42, or 84 d. They found, that after 42 d, off-
flavors scores were decreased. Regardless of the exact number of days required to
see improved flavor profiles, it is generally accepted that feeding a high-energy ration
prior to harvest will increase consumer acceptability by decreasing "grassy" off-flavors
associated with forage finished beef.

Economic Considerations for Realimentation Programs

Even though literature confirms benefits of cull cow realimentation programs from
a meat quality standpoint, producers must take into account economic considerations.
One of the most important considerations is selecting the right type of cow for
realimentation. Cows placed in realimentation programs need to be healthy and open
(non pregnant). Cows also need to be in thin to moderate condition to allow greater
potential for compensatory gain.   Even though all ages and breeds will show some
response to feeding, not all cows are well suited for profitable realimentation programs.
Often, greater variability in performance and carcass characteristics is observed in older
cows making it more profitable to select younger cows for feeding programs. Harborth
(2006) reported that cows less than 6 years of age had greater feedlot performance
than older cows. Additionally, Continental and British breeds typically offer more growth
potential and will result in greater monetary returns than Brahman-influenced cattle
(Woerner, 2010).

A second major consideration is cost of gain and economic returns on marketing
fed-cows. The primary cost associated with cull cow realimentation is the cost of feed.
It is estimated that during realimentation, cows consume 2.5% or more of their body
weight as DM (Wright, 2005). The cost of feeding will greatly depend on grain prices,
feed efficiency of selected cows, and number of days on feed. However, reports from
2005 through 2007 calculated the cost per pound of gain to be somewhere between

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$0.40 to $0.50 (Wright, 2005; Carter and Johnson, 2007). Additional items that must be considered in cost of feeding are yardage charges associated with commercial feed yards and increased freight/fuel costs for transporting heavier cattle.

Premium white cows almost always yield a premium price and increased weights of carcasses from fed-cows can improve returns. In July of 2008, premium white cow carcasses averaged $116 to $128/cwt while 500 lb and up breaker carcasses only ranged from $100 to $119/cwt (USDA, 2008). Therefore, if the cost of gain is reasonable and marketing is timed at seasonal highs, feeding cull cows can yield significant economic benefit. However, producers must conduct a budget analysis to evaluate the most profitable marketing/management decisions for cull cows. In addition, producers must have access to and have marketing arrangements for fed cows before initiating a fed-cow program.

ANABOLIC STEROID IMPLANTS

Anabolic steroids were the first compounds to be used on a commercial scale to increase gains, feed efficiency, and carcass yields in livestock (Sillence, 2004). There are two types of anabolic hormones used in cattle implants – androgenic, which mimic testosterone, and estrogenic, which mimic estrogen. Several combination implants containing both androgenic and estrogenic compounds are also available.

Mode of action

The mode of action of anabolic steroid implants can be attributed to both increased protein synthesis and decreased protein degradation. Steroid hormones can elicit a response primarily via two mechanisms classified as either genomic or rapid signaling responses. The genomic response influences growth by binding intracellular steroid receptors that effect the regulation of gene transcription factors and protein synthesis (Beato et al., 1996). Rapid signaling responses can occur within seconds to minutes of cell stimulation (Prossnitz et al., 2008). This mechanism involves steroid ligands binding growth factor receptors and transmembrane G-protein couple receptors. Anabolic steroids are known to increase levels of IGF-I (Frey et al., 1994; Johnson et

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al., 1996; Johnson et al., 1998; Dunn et al., 2003; White et al., 2003). Insulin growth factor-I is especially important in postnatal skeletal muscle growth because it is a progression factor capable of stimulating satellite cell proliferation and cell differentiation (Allen et al., 1979). Satellite cells provide a necessary supply of nuclei to support increased protein synthesis. In addition to influencing satellite cells, some anabolic compounds can block progenitor cells from the adipogenic pathway (Johnson and Chung, 2007).

Use in cull cow realimentation

Anabolic steroid implants have been thoroughly researched in young steer and heifer feeding systems. A smaller proportion of studies have looked at implants in mature cow realimentation programs. No implant currently carries approval from the Food and Drug Administration (FDA) for specific use in cull cows. However, FDA generally holds the view that implants approved for feedlot heifers may be used in cull cows that are placed in a feedlot setting prior to harvest.

Implanting cows with androgenic implants tends to enhance performance over estrogenic implants. Corah et al. (1980) found that cows grazing fescue pasture and implanted with zeranol had an 11% increase in weight gains during a 59 d period compared to non-implanted cows. In contrast, Price and Makarechian (1982) reported that mature cows implanted with 36 or 72 mg of zeranol showed no improvements in growth rates or carcass characteristics. Matulis et al. (1987) found no significant improvements in growth performance when cows were implanted with Synovex-H, a combination implant containing 200 mg testosterone propionate and 20 mg estradiol, and fed a high-energy ration for 56. Similar results were seen by Faulkner et al. (1989) when using Synovex-H in cull cow realimentation. They found few significant differences in growth efficiency, carcass traits, carcass composition, and sensory properties between implanted and non-implanted cows fed for 42 or 84 d. Pritchard and Berg (1992) studied the effects of implanting cull cows with Finaplix-H, an implant containing 200 mg of trenbolone acetate. They found that implanted cows had similar ADG and lower DM intake, but greater G:F than non-implanted cows. In a comprehensive study comparing no implant, Finaplix-H, Synovex-H, or a combination of

the two implants, Cranwell et al. (1996b) reported that cows implanted with both implants (Synovex-H + Finaplix-H) had advantages in final weight, ADG and G:F compared to non-implanted controls. Using contrast statements, these researchers also determined that implanted cows (combined data from all three implant treatments) had heavier HCW, larger LM areas, and improved yield grades compared to non-implanted controls. In a follow-up publication, Cranwell et al. (1996a) reported that implanting did not influence visual carcass quality traits, but that implanted cows had increased collagen solubility and improved sensory panel ratings for LM tenderness. The improvement in tenderness of steaks from implanted cows was likely due to increased protein turnover and thus higher quantities of newly synthesized, less crossed-link collagen. Harborth (2006) evaluated the use of Revalor-200, a combination implant with 200 mg of trenbolone acetate and 20 mg estradiol, and found that implanted cows had increased ADG, and a tendency for increased HCW, DP, and LM areas compared to non-implanted controls. In summary of these studies, combination steroid implants that contain both androgenic and estrogenic compounds can enhance cull cow realimentation by improving performance, carcass characteristics and some aspects of sensory traits.

β-ADRENERGIC AGONISTS

Numerous pharmaceutical companies have developed β-AA compounds for potential use in the livestock industry. β-adrenergic agonists such as clenbuterol, cimaterol, and $_L$-644,969 have been shown to increase ADG, improve feed efficiency, and increase LM area when fed to cattle (Mersmann, 1998). However, currently only two β-AA are approved for use in cattle. Ractopamine-HCl (RH; Optaflexx®, Elanco Animal Health) was approved for use in feedlot cattle in 2003, and zilpaterol-HCl (ZH; Zilmax®, Intervet) was approved in 2006. Both RH and ZH belong to a class of compounds called phenethanolamines and are administered as feed ingredients in finishing rations. Phenethanolamines have been used effectively in human medicine for many years to treat asthma and cardiac irregularities. However, the repartitioning

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effects of β-AA and their ability to influence skeletal muscle was not discovered and utilized by the livestock industry until the 1970s.

Mode of action

Unlike steroid implants, β-AA enhance muscle growth without a change in nuclei number, but rather through direct regulation of protein synthesis and degradation. The physiological β-AA are norepinephrine and epinephrine. Both physiological β-AA and synthetic compounds elicit a response via adrenoreceptors. Adrenergic receptors belong to a family of cell-surface receptors termed guanine nucleotide binding protein-coupled receptors (GPCR) and elicit a response via guanine nucleotide binding proteins (G-proteins). All GPCR have a similar structure with approximately 400 amino acids that form seven transmembrane α-helices with three extracellular loops, and three intracellular loops (Morris and Malbon, 1999). The G-protein complex is comprised of three subunits Gα, Gβ, and Gγ. The Gβ and Gγ subunits form a dimer that is bound to the intracellular plasma membrane. In an inactive state, the Gα subunit is bound to the Gβγ dimer. When a ligand binds at the 7th transmembrane domain of the receptor, the Gα subunit dissociates from the Gβγ dimer releasing GDP. Subsequently, GTP binds the Gα-subunit and adenylate cylcase is activated. Adenylate cyclase catalyzes the reaction of ATP to cAMP. Then, cAMP binds phosphokinase A (PKA), allowing the phosphorylation of numerous proteins that in return influence protein and fat synthesis/degradation (Lynch and Ryall, 2008).

Adrenergic receptors can be divided into two subtypes, α and β. The β-ARs are the most abundant AR found in muscle. In a detailed review by Lynch and Ryall (2008), several direct and indirect mechanisms of β-AR signaling pathways were described. The most broadly researched signaling pathway is the phosphorylation of intracellular proteins mediated by phosphokinase A (PKA). Phosphorylation via PKA activates some intracellular proteins and inactivates others. Several key intracellular proteins that are influenced by phosphorylation include hormone sensitive lipase, cAMP response binding protein, and acetyl-CoA carboxylase. The phosphorylation of hormone sensitive lipase leads to increased adipocyte triacylglycerol degradation. When cAMP response-element-binding protein is phosphorylated, the transcription of numerous genes is

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influenced. Acetyl-CoA carboxylase is inactivated by phosphorylation, which leads to a decrease in long-chain fatty acid biosynthesis (Mersmann, 1998).

Indirect mechanisms of β-AR signaling have also been studied. Agonists binding to receptors yield increased blood flow, which may boost the delivery of substrates and energy sources needed for protein synthesis while also providing a medium to transport fatty acids away from tissues (Mersmann, 1998). Another theory is that β-AR signaling may alter circulating concentrations of endocrine substances such as insulin, plasma thyroid hormones, and endogenous plasma catecholamines (Beermann et al., 1987).

Distribution of β-adrenergic receptors in cattle

β-adrenergic receptors are found on the surface of most all mammalian cells (Mersmann, 1998). Three β-AR subtypes have been identified as β_1, β_2, and β_3. The distribution of these subtypes varies among species and specific tissues. Research conducted by Sillence and Matthews (1994) is the most widely cited source of β-AR densities in bovine skeletal muscle and adipose tissue. They reported that bovine skeletal muscle and adipocyte cells contain predominately β_2-AR and lower densities of β_1- and β_3-AR. It is thought that β_3-AR may primarily reside in the few brown adipocytes remaining in amongst the white adipose tissue of adult bovines.

β-adrenergic receptors can be affected by desensitization, defined as the attenuation of response despite continued presence of the stimulus (Mills, 2002). Primarily two types of desensitization can occur: acute uncoupling and chronic down-regulation. Uncoupling is typically associated with the phosphorylation of the receptor by protein kinase (PKA and PKC) as well as tyrosine kinase. Phosphorylation interferes with the Gs mechanism, and the affinity of receptors for β-AA is greatly reduced once phosphorylation has occurred (Mills, 2002). Chronic down-regulation develops more slowly than acute uncoupling and is a reduction in the number of β-AR. The rate of down-regulation depends on potency and efficacy of the β-AA (Mills, 2002). It is because of these cellular regulation mechanisms that commercially available β-AA are only approved to be used during the later stages of feeding. Extended exposure of cellular systems to β-AA would lead to a down-regulation and reduced responsiveness to the compound.

Although little research is available for skeletal muscle, it is generally thought that function of β-AR decreases with advancing age in man and animals (Elfellah et al., 1989). Some researchers have attributed reduced function to a reduction in receptor density, while others have found decreased affinity of receptors for β-AA ligands (Elfellah et al., 1989). Currently, no published literature describes changes in β-AR densities or affinity for ligands as cattle mature.

Influence of β-adrenergic agonists on mRNA

Several researchers have studied the influence of β-AA on mRNA levels to gain a better understanding of the β-AA mode of action at a cellular level. Walker et al. (2007) reported that RH supplementation (200 mg·head^{-1}·d^{-1} for 28 d) in Holstein steers decreased β$_1$- and β$_3$-AR mRNA in the LM. However, Winterholler et al. (2007) found a tendency for β$_2$-AR mRNA in the *semimembranosus* to increase with RH supplementation in yearling steers. They went on to determine that bovine primary muscle cultures showed an increase in β$_2$-AR mRNA level during differentiation due to RH treatment. In agreement with these findings, Sissom et al. (2007) reported a tendency for β$_2$-AR mRNA in the *semimembranosus* to be increased when RH was supplemented to heifers. Gonzalez et al. (2008) published data that similar mRNA responses are observed in mature cows. When cull cows were supplemented with varying levels of RH, β$_2$-AR mRNA levels were not diminished in response to RH in the LM or *semimembranosus* muscle. Instead, there was a trend for β$_2$-AR mRNA concentrations to be increased by RH supplementation. It is thought that β-AR are regulated by endogenous catecholamines. In rats, Sillence et al. (1995) demonstrated that β$_2$-AR density can be up-regulated by β-antagonists and agonists. It is thought that, even though RH is primarily a β$_1$-AA, it may bind weakly to β$_2$ receptors resulting in an up regulation of β$_2$-AR.

Ractopamine-HCl

Ractopamine-HCl is approved for use in the swine industry under the trade name Paylean® and in cattle under the trade name Optaflexx®. Optaflexx® is labeled for use in cattle fed in confinement for harvest during the last 28 to 42 d of feeding at a level of 70 to 430 mg·head^{-1}·d^{-1} to increase rate of weight gain, improve feed efficiency, and

increase carcass leanness (FDA, 2003). In general, it is thought that RH preferentially binds to β_1 receptors, but can also bind β_2 receptors to elicit a response (Moody et al., 2000).

Use in Young Cattle

Numerous studies have evaluated the influence of RH on feedlot performance and meat quality in young steers and heifers. In a study comprised of 25 experiments and 880 steers, the effects of feeding varying levels (100, 200, or 300 mg·head^{-1}·d^{-1}) of RH in feedlot rations for the last 28 to 42 d of finishing, Schroeder et al. (2005a) found that RH increased ADG, FE and HCW for all treatment levels compared to controls. Dressing percent was improved when 200 or 300 mg·head^{-1}·d^{-1} was fed. Additionally, Schroeder et al. (2005b) reported that LM area was improved by feeding all levels of RH, and yield grade was improved when RH was fed at 300 mg·head^{-1}·d^{-1}. Marbling score, FT, muscle color, firmness, and texture were not influenced by feeding RH. Loe et al. (2005) evaluated RH supplementation in a commercial feedlot setting and reported that feeding 200 mg·head^{-1}·d^{-1} for approximately 29 d improved G:F and increased HCW while having no influence on USDA quality grade. Winterholler et al. (2007) found that feeding steers 200 mg·head^{-1}·d^{-1} of RH for 28 d increased ADG by 4.6% and feed efficiency by 3.8%. They also reported that RH administration did not alter DP, quality grade, or yield grade. Carcass weights were increased by 8 kg and LM area was increased by 1.74cm^2 compared to control steers.

Similar to data observed in steers, Schroeder et al. (2005c) reported that heifers fed RH (100, 200, or 300 mg·head^{-1}·d^{-1}) for 28 or 42 d had greater ADG and improved G:F compared to controls. They also reported that heifers fed 200 mg·head^{-1}·d^{-1} had heavier HCW and heifers fed 300 mg·head^{-1}·d^{-1} had larger LM areas. Walker et al. (2007) reported that feeding RH to heifers improved ADG, G:F, and HCW compared to controls not fed RH. Quinn et al. (2008) found fewer improvements in performance and carcass traits than previous researchers when heifers were fed 200 or 300 mg·head^{-1}·d^{-1} of RH for either 28 or 42 d, but concluded that there appears to be no benefit in feeding higher levels of RH for 42 d compared with continuous feeding of 200 mg of RH for 42 d.

Because β-AA repartition nutrients from adipose tissue disposition toward skeletal muscle growth, much concern exists regarding the influence that β-AA have on marbling scores and USDA quality grades. In a comprehensive study conducted by Elanco, RH dose (100, 200, and 300 mg·head^{-1}·d^{-1}) and feeding duration (28 and 42 d) had no influence on marbling score (Schroeder et al., 2005a). Several other studies reported similar findings with no difference in marbling scores in RH supplemented steers and heifers compared to controls (Winterholler et al., 2007; Quinn et al., 2008) In contrast, Grueber et al. (2007) reported that steers fed RH had reduced marbling scores compared to control steers. However, the alteration in marbling score was not sufficient to influence distribution of USDA quality grades.

In an FDA report, feeding steers 100 and 200 mg·head^{-1}·d^{-1} of RH for 28 and 42 d did not influence WBSF values or sensory panel ratings for tenderness, juiciness, beef flavor, or off-flavor of LM steaks. However, feeding 300 mg·head^{-1}·d^{-1} of RH increased WBSF measurements and led to less desirable sensory panel ratings for tenderness (FDA, 2003). Avendano-Reyes et al. (2006) reported that feeding 300 mg·head^{-1}·d^{-1} of RH increased WBSF values compared to controls. In contrast though, Quinn et al. (2008) reported that heifers supplemented with 200 mg·head^{-1}·d^{-1} of RH for 28 d prior to harvest produced LM steaks with similar WBSF values as control heifers. Gruber et al. (2008) also found that feeding 200 mg·head^{-1}·d^{-1} of RH to steers for the final 28 d of finishing caused LM WBSF values to increase by 0.38 kg and sensory ratings for tenderness, juiciness, and beef flavor to decrease compared to control samples. Even though tenderness improved with increased postmortem aging, the effects of feeding RH were not counteracted by aging LM samples for 21 d. In studies utilizing more potent β-AA, it is expected that meat toughness will be increased due to reduced postmortem proteolysis (Geesink et al., 1993).

Use in Mature Cows

A limited number of studies have looked at the impact of feeding RH to mature beef cows. Harborth (2006) evaluated the effects of implanting mature cows with Revalor-200 and feeding 300 mg·head^{-1}·d^{-1} of RH for the final 28 d of a 60 d feeding period. In that study, no major improvements were observed in feedlot performance or

carcass value of cows fed RH alone or in combination with Revelor-200. In a dose titration study where cull cows were fed 100, 200, or 300 mg·head^{-1}·d^{-1} of RH, no improvements were seen in feedlot performance (Carter et al., 2006) or carcass characteristics (Dijkhuis et al., 2008). Holmer et al. (2009a) reported no significant differences in feedlot performance, carcass traits, muscle yields or muscle proximate analysis between RH supplemented cows and cows fed a high-energy ration without supplementation. In addition, Holmer et al. (2009b) reported that feeding RH had no adverse affects on sensory proprieties of 10 muscles compared to feeding a concentrate ration without RH supplementation. In fact, *psoas major* WBSF values were decreased following RH supplementation.

Even though mature cow performance, carcass, and muscle characteristics undergo few changes with RH supplementation, research indicates that, on a cellular level, RH is eliciting a response. Gonzalez et al. (2008) reported in a follow-up publication to the dose titration studies conducted by Carter et al. (2006) and Dijkhuis et al. (2008), that in the *semimembranosus* and *vastus lateralis* muscles, the percentage of Type I fibers decreased and the percentage of Type IIA fibers increased as RH dose increased. In the LM, fiber type distribution was altered by feeding at least 100 mg·head^{-1}·d^{-1} of RH, but further changes were not observed with increased dose. For the *infraspinatus* muscle, 200 mg·head^{-1}·d^{-1} of RH altered fiber type distributions, but further changes were not observed by increasing dosage to 300 mg·head^{-1}·d^{-1}. Similar transitions in fiber type distributions have been noted in rats and mice (Lynch and Ryall, 2008). Type I muscle fibers are slow-twitch, oxidative, and have low glycolytic capacity. Type II fibers are fast-twitch and have higher glycolytic capacity. Data indicate that rats selected for rapid postnatal growth have more fast-contracting muscle fibers (Sosnicki, 1987). In addition, double-muscled cattle and callipyge lambs typically have a greater proportion of fast glycolytic muscle fibers (Lefaucheur and Gerrand, 2000). Therefore, the transition of Type I (oxidative) to Type II (glycolytic) fibers reported in cows supplemented with RH should translate into potential for more growth. However, it is unclear why transitions in muscle fiber types due to RH supplementation do not translate into significantly enhanced muscle growth in mature cows.

Zilpaterol-HCl

Zilpaterol-HCl was approved for use in South Africa (1997), followed by Mexico (1999), the United States (2006), and more recently in Canada (2009). Zilpaterol-HCl is marketed under the trade name Zilmax® and is labeled for use in cattle fed in confinement for harvest during the last 20 to 40 d of feeding at 8.3 mg/kg (100% DM basis) to improve body mass gain and feed conversion while improving carcass leanness by reducing fat deposition. A 3-d withdrawal is required prior to harvest. In general, it is thought that ZH preferentially binds to β_2 receptors (Moody et al., 2000).

Use in Young Cattle

In a study conducted in South Africa prior to Zilmax® approval in the United States, Plascencia et al. (2008) reported that feeding yearling, crossbred steers 6 mg/kg of ZH in a 42-d finishing trial improved ADG and G:F by approximately 28% compared to non-supplemented controls. Avendano-Reyes et al. (2006) observed that steers had 26% greater ADG when supplemented with ZH. In more recent studies conducted in the United States, Vasconcelos et al. (2008) and Elam et al. (2009) reported that ADG and G:F were optimized in steers supplemented with ZH for 20 to 40 d compared to non-supplemented controls. Montgomery et al. (2009) reported that feeding ZH at 8.3 mg/kg of feed improved ADG and G:F by 36 and 28%, respectively, in steers and by 18 and 21%, respectively, in heifers.

Some carcass traits are also improved with ZH supplementation. Plascencia et al. (2008) found that supplementation with ZH increased LM area, but no differences were reported for FT or internal fat measures. Avendano-Reyes et al. (2006) reported that feeding ZH for 33 d increased finished body weight by 19.5 kg, increased HCW, improved DP, increased LM area, and a tended to reduce12[th]-rib fat. Similarly, Vasconcelos et al. (2008) reported that final body weights were increased by 9 kg, HCW was increased by 15 kg, dressing percentage was improved by 2 percentage points, and LM area measured 9.6 cm^2 larger. A major benefit from a commercial economic standpoint is the ability of ZH to increase carcass cutability and meat yields. Rathmann et al. (2009) reported that supplementation of ZH increased lean yield in 22 of 33 subprimals evaluated. Hilton et al. (2010) also reported that saleable weight and yield

percentages of boneless, closely trimmed subprimals, other cuts, and trim were increased by ZH supplementation for 20 to 40 d. Increased muscling often comes at the expense of carcass fat. Vasconcelos et al. (2008) found that carcasses from cattle fed ZH were leaner with 0.22 cm less 12th-rib fat, had lower %KPH, decreased yield grades, and reduced marbling. Montgomery et al. (2009) also found that, along with increased HCW, DP and LM area, quality grade and yield grade were decreased. It is hypothesized that feeding ZH enhances muscle growth by repartitioning nutrients from fat deposition towards protein synthesis. In some cases, this may negatively affect USDA quality grades and, therefore, must be addressed in individual feedlot marketing programs.

Because protein degradation is decreased with the supplementation of β-AA, tenderness is a concern when feeding these growth promoting agents. Avendano-Reyes et al. (2006) reported that supplementation with ZH increased WBSF values. In a summary of numerous ZH studies, Delmore et al. (2010) discussed that WBSF values are increased to a greater extent when ZH is supplemented for greater than 20 d (Brooks et al., 2009; Leheska et al., 2009) and that postmortem aging for 21 d reduces differences in WBSF values greater than aging for only 7 or 14 d postmortem (Hilton et al., 2009; Kellermeier et al., 2010). Effects of ZH supplementation on tenderness of different muscle by gender combinations has been shown to differ (Claus et al., 2010). Supplementation of feedlot rations with ZH for 20, 30 or 40 d increased WBSF of steer LM and heifer *triceps brachii*; however, supplementation with ZH for 30 or 40 d increased WBSF of heifer LM muscles. In this study, aging muscles at least 21 d mitigated toughness for steer LM and heifer *triceps brachii*, but not heifer LM and *gluteus medius*. Therefore, even though ZH decreases tenderness, supplementing for only 20 d and aging steaks for at least 21 d can reduce the risk but not eliminate the possibility of steak toughness.

Use in Mature Cattle

Very few studies have evaluated the effectiveness of ZH in cull cow realimentation programs. Neill et al. (2009) conducted a detailed study that compared grass-feeding systems to cull cow realimentation programs that consisted of 1)

concentrate ration only, 2) Revalor-200 implant plus concentrate ration, 3) ZH only, and 4) implant plus ZH. For this study, ZH was fed for the last 30 d of a 70-d feeding period. Their results indicated few improvements in feedlot performance due to implanting or supplementation with ZH. In general, carcass traits were improved when a concentrate ration was fed, regardless of implant or ZH treatment. The only carcass trait significantly influenced by ZH supplementation was LM area. Additionally, realimentation improved subprimal yields, but ZH supplementation rarely translated into greater subprimal weights than concentrate feeding alone or in combination with implants. Sensory panelists rated LM steaks from implanted plus ZH supplemented cows as tougher and to contain more connective tissue than concentrate-fed only, implanted and concentrate-fed, and grass-fed cows. In addition, LM steaks from ZH treated cows had the highest WBSF values (Hutchison, 2007). In a recent study conducted in South Africa, Strydom and Smith (2010) evaluated supplementing cull cows with ZH for 20, 30, for 40 d. They reported that supplementation with ZH improved G:F, DP, and conformation scores. In addition, the percentage of fillet and thick flank meat was increased when ZH was supplemented for 30 or 40 d. However, in contrast to previous work, they found no difference in tenderness between ZH supplemented and control cows. A recent abstract reported more promising results in regards to ZH supplementation in mature cow feeding programs. Gasch et al. (2010) reported that feeding 8.33 mg/kg of ZH for 20 d increased ADG, carcass yield, LM area, and value of saleable yield compared to non-implanted controls.

It can be concluded that β-AA do not have as profound of an impact on feedlot performance or meat characteristics in mature cows as they do in young steers and heifers. This lack of response may be due to decreased β-AR function in older animals. Both reduced density of β-AR and deceased sensitivity of receptors to agonists have been reported in older humans and animals (Elfellah et al., 1989).

Additive Effects of Steroidal Implants and β-AA

Several researchers have explored the potential for additive effects by implanting cattle with steroidal implants prior to β-AA supplementation. Walker et al. (2007) reported that heifers implanted with Revalor-H 60 d prior to RH supplementation had

improved ADG, carcass-adjusted ADG, and carcass-adjusted G:F. Additionally, Sissom et al. (2007) found that heifers implanted with Revelor-200 or Finaplix-H 154 and 96 d prior to RH supplementation had increased growth rate and feed efficiency. Additionally, research has explored additive effects of steroidal implants and ZH. Baxa et al. (2010) reported that when steers were implanted with Revalor-S prior to ZH supplementation, additive improvements were observed in ADG, G:F, HCW, LM area, and dressing percentage compared to individual treatment with Revalor-S or ZH. Additionally, additive decreases in 12th-rib fat depth, marbling score, and KPH were observed in steers implanted prior to ZH supplementation compared to individual treatments of implant or ZH. In a mature cow study, Neill et al. (2009) found that ZH and Revalor-200 worked synergistically to increase HCW, LM area, and total subprimal weight. These researchers postulated that to maximize muscle accumulation in cull cows, an anabolic implant must be administered prior to β-AA supplementation in order to stimulate quiescent satellite cells to proliferate to increase the amount of DNA available to sustain muscle hypertrophy initiated by β-AA. The additive effects of implants and β-AA may be due to anabolic steroids eliciting a biological response that synthesizes the "machinery" necessary for muscle growth, and β-AA then improving the efficiency with which that "machinery" is capable of accumulating protein (Baxa, 2008).

TENDERNESS

The Basics

Meat tenderness has been reported as the most important factor affecting consumer satisfaction of beef palatability (Dikeman, 1987; Savell et al., 1987). In a review article, Koohmaraie and Geesink (2006) discussed the three factors that determine meat tenderness: background toughness, the toughening phase and the tenderization phase. Marsh and Leet (1966) defined background toughness as the "resistance to shearing of the un-shortened muscle" and attributed most background toughness to connective tissue. The toughening phase occurs during the conversion of muscle to meat when sarcomeres shorten during rigor development (Koohmaraie,

1994). The tenderization phase is a result of proteolysis of myofibrillar and myofibrillar-associated proteins during postmortem storage. Of all the phases, the most highly variable from product to product is the tenderization phase due to variation in both rate and extent of postmortem proteolysis (Koohmaraie and Geesink, 2006)

Background toughness (Connective tissue)

Connective tissue comprises approximately 1 to 4% of the dry weight of most muscles (Purslow, 2005). Collagen is the primary protein found in connective tissue components of muscle. The amount and solubility of collagen found in a given muscle can be related to muscle type, breed, and animal age. For the purpose of this review, animal age will be the only factor discussed. Total collagen content is relatively constant in animals of various ages (Smith and Judge, 1991). However, studies have demonstrated that increased animal age results in decreased collagen solubility and, in return, tougher meat. The change in collagen solubility throughout animal maturity is attributed to increased thermal and mechanical stability via increased cross-linking (Bailey and Light, 1989). During stages of muscle growth, collagen must be synthesized to aid in structural support of muscle. Newly synthesized collagen has fewer stabilized cross-links and should be more heat-labile (Etherington and Sims, 1981). Several researchers have demonstrated that feeding mature cows prior to harvest and using growth promoting agents can increase muscle growth, increasing the amount of newly synthesized collagen resulting in enhanced meat tenderness (Cranwell et al., 1996a; Boleman et al., 1996).

Toughening Phase

During rigor mortis, longitudinal and lateral contractions occur due to a fall in pH and the attachment of myosin heads to the actin filaments (Tornberg, 1996). When muscle temperature drops below 15°C before rigor, the sarcoplasmic reticulum becomes increasingly unable to bind calcium. Because ATP is still present in the muscle, the muscle contracts to a maximum level causing filaments to slide together (Savell et al., 2005). Most cattle are harvested in commercial processing plants that chill all carcasses at the same temperature regardless of carcass size or amount of external fat. Fat thickness is known to contribute to the extent of cold shortening in beef

(Dolezal et al., 1982). Increased external fat was found to improve tenderness by allowing carcasses to chill more slowly and increase enzyme activity (Smith et al., 1976). Cow carcasses, especially those from cows not realimentated, have lighter carcass weights and reduced fat thicknesses (Neill et al., 2009). Therefore, some toughness associated with mature beef carcasses can be linked with cold shortening from leaner carcasses chilling at a rapid rate prior to the onset of rigor. Additionally, many of the studies discussed previously in this review reported that the use anabolic steroid implants and β-AA reduce fat thickness. This reduction in external fat may contribute to toughness associated with meat from livestock treated with growth promoting compounds.

Tenderization Phase

Throughout postmortem storage, proteins such as troponin-I, troponin-T, desmin, nebulin, and titin are degraded, which weakens the myofibers in muscle (Koohmaraie and Geesink, 2006). The degradation of these proteins is primarily caused by the calpain enzyme system. Evidence of calpain mediated tenderization has been widely established via *in vitro* and *in vivo* studies. A second enzyme that can play a major role in postmortem tenderization is calpastatin, the endogenous inhibitor of μ and m-calpain (Koohmaraie and Geesink, 2006). Numerous studies discussed previously found that growth promotants, such as β-AA and implants, have potential to enhance muscle growth by reducing protein degradation. This same mechanism effects meat tenderness through elevations of calpastatin in muscle undergoing increased muscle hypertrophy. Calpastatin is elevated in the live animal resulting in decreased protein degradation, thus increased muscle growth. The increased levels of calpastatin carry over into postmortem muscle inhibit calpains from carrying out postmortem proteolysis (Koomaraie et al., 2002).

ENHANCEMENT WITH CALCIUM LACTATE

It is well documented that beef from older cattle is tougher, typically drier, and has a mealiness residue compared to meat from yearling steers and heifers (Shorthose and Harris, 1990). In addition, it has been shown that some growth promoting

treatments, such as implants and β-AA, can decrease meat tenderness. Various methods such as natural aging, the use of enzymes, electrical stimulation, carcass suspension, and injection enhancement with various substances have been employed to reduce negative attributes of beef sensory traits (Hoffman, 2006). Substantial attention has been given to enhancement with calcium salts. Two mechanisms are responsible for the effects of calcium ions on meat tenderization. The first, and most widely accepted mechanism, is activation of calcium-dependent proteases found in skeletal muscle (Koohmaraie et al., 1986; Whipple and Koomaraie, 1991). A second mechanism is a non-enzymatic salting-in that leads to protein solubilization (Hattori and Takahashi, 1979; Takahashi et al., 1987; Taylor and Etherington, 1991). Lawrence et al. (2003) reported that calcium-activated enzymatic activity accounted for approximately 70% of tenderization and non-enzymatic salting-in accounted for approximately 30% of tenderization when the LM was enhanced with calcium ions at 72 h postmortem and allowed to age to 15 d postmortem.

Even though injection enhancement with calcium salts is beneficial in regards to tenderness, some forms of calcium, such as calcium chloride, can cause negative sensory and color attributes. Researchers have reported that calcium chloride induces bitter, metallic, and livery off-flavors (Morgan et al., 1991). In addition, it has been shown that calcium chloride can induce muscle darkening, cause faster discoloration, and increase aerobic plate counts (Wheeler et al., 1993; Kerth et al., 1995).

Lawrence et al. (2003) conducted a detailed study to compare the effects of calcium salts on beef LM quality. Strip loins were injected with varying concentrations (0.1 M to 0.3 M) of calcium salts (calcium ascorbate, calcium chloride, and calcium lactate), vacuum tumbled, and allowed to age until 14 d postmortem. Calcium lactate injection inhibited microbial growth, as quantified by aerobic plate counts, more than calcium ascorbate and calcium chloride. As for tenderization effects, all calcium salts performed equally. Sensory panel ratings indicated that calcium lactate injection yielded more intense beef flavor than calcium chloride or calcium ascorbate. Furthermore, beef flavor intensity was rated as higher for 0.1 M concentrations than 0.3 M concentrations.

In a later study, Lawrence et al. (2004) concluded that calcium lactate solutions provide better initial color and color stability throughout retail display than phosphate and salt solutions. Kim et al. (2006) described lactate as a "color stabilizer" because lactate can minimize whole-muscle cut surface discoloration during storage and display (Lawrence et al., 2003; Lawrence et al., 2004; Kim et al., 2006; Knock et al., 2006). Recent research suggested that the underlying mechanisms for lactate-modulated beef color stability is that lactate promotes redox stability via direct interactions with myoglobin and indirect interactions with lactate dehydrogenase (Kim et al., 2006; Kim et al., 2009).

It can be concluded that injection enhancement with 0.1 M calcium lactate improves tenderness while minimizing negative flavor attributes and without sacrificing color or microbial shelf-life. Injection enhancement with calcium ions has potential to improve beef tenderness in meat from mature cows and cattle treated with growth promoting compounds. However, calcium lactate is the salt form that can improve tenderness, yet minimize the potential negative influence that enhancement may have on color stability and flavor profiles.

COLOR STABILITY OF COW BEEF

Meat color serves as an indicator of freshness and wholesomeness to consumers and heavily influences purchasing decisions. Numerous factors such as ration, growth rate, age, gender, genetics, and muscle properties can all influence meat color. Beef from mature cows can be associated with meat color disadvantages. Typically, as cattle mature, myoglobin content increases which causes darker colored lean (Romans et al., 1965). To support this, Sawyer et al. (2004) observed that lean color tends to darken as cattle age. Recently, Patten et al. (2008) reported cow *gluteus medius*, *longissimus*, *triceps brachii*, *psoas major*, *rectus femoris* and *tensor facia latae* were darker (lower L*) than the same muscles from steers. Xiong et al. (2007) evaluated the oxidative stability of muscles from mature cows. Although few differences due to age were observed in color and pigment oxidation, lipid oxidation was affected by cow age. Ground beef patties from non-realimented10 to 12 yr-old cows were most

susceptible to lipid oxidation, followed by the 6 to 8 yr-old group, and the 2 to 4 yr-old group was the least susceptible.

The energy density of the ration also has a profound influence on beef color. Lean color can differ due to grain-feeding versus forage-feeding of cattle. Watanabe et al. (1993) reported that carcass pH of forage-fed cattle tends to be higher than the pH of grain-fed cattle. Additionally, Vestergaard et al. (2000) suggested that forage-based rations promote oxidative muscle metabolism instead of anaerobic muscle metabolism, thus leading to reduced glycogen storage. The consequence of increased oxidative muscle metabolism and reduced glycogen storage is smaller postmortem pH decline. Ration and feeding duration affects the color of meat from realimentated cows. Concentrate-fed cows were found to have brighter, redder lean than forage-fed cows (Price and Berg, 1981). Boleman et al. (1996) found that cows fed a high-energy, high-protein ration for 28, 56, or 84 d yielded carcasses with brighter, redder lean color than cows fed a low-energy, low-protein ration for the same durations. Duration of feeding can also affect meat color. Cull beef cows fed for 56 d had redder LM color than cull beef cows fed for only 28 d (Cranwell et al., 1996a).

Feeding β-AA to cattle also might alter beef color by causing paler lean color. This has been attributed to reduced heme pigmentation and to a larger proportion of fast-twitch glycolytic fibers (Beerman et al., 1987; Wheeler and Koohmaraie, 1992). Quinn et al. (2006) reported no differences in lightness, redness, or yellowness of longissimus steaks from beef heifers fed RH for 28 d compared to control heifers. However, in contrast, Holmer et al. (2009) reported that cull cows supplemented with RH had lower L* values, indicating darker muscle color, than non-supplemented controls. Studies evaluating ZH have suggested that supplementation provides no detrimental effects on lean color and may actually improve beef color stability. Hilton et al. (2009) reported that LM steaks from young cattle supplemented with ZH for 30 d maintained a brighter cherry red color throughout retail display. In contrast, Hutchison (2007) reported that discoloration patterns of LM steaks from cull cows supplemented with ZH were not different than control treatments.

SUMMARY

Meat from mature cows is typically of poorer quality than meat from younger steers and heifers. Realimentation programs can improve cow meat characteristics and palatability while also increasing lean meat yields and cow value. Feeding a high-concentrate ration for 50 to 100 d can efficiently improve carcass traits and meat sensory properties. Even though growth promoting technologies, such as anabolic steroid implants and β-AA, are less effective in mature cows than young cattle, they may still be valuable in enhancing some aspects of cow performance, carcass traits, or meat sensory characteristics.

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Chapter 3 - Effects of sequential feeding of β-adrenergic agonists on cull cow performance, carcass characteristics, and mRNA relative quantities

ABSTRACT

The objectives of this study were to determine the effects of supplementation with a single or sequence of β-adrenergic agonists (β-AA) on cow performance, carcass characteristics, mRNA expression, and economics of production of cull cows implanted and fed a concentrate ration. Sixty cull cows were implanted with Revalor®-200 and assigned to 1 of 4 treatments: C = fed a concentrate ration; RH = supplemented with ractopamine-HCl (RH) for the last 25 d; ZH = supplemented with zilpaterol-HCl (ZH) for the last 20 d; RH + ZH = supplemented with RH for 25 d followed by ZH for the last 20 d. All cows were fed the concentrate ration for 74 d. For the ZH and RH + ZH treatments, cows had a 3 d withdrawal from ZH prior to harvest. Cows were allotted with 5 cows per pen and 3 replicate pens for each treatment. Live weights were collected on d 1, 24, 51, and 72. Muscle biopsies from the LM were collected on d 24, 51, and at harvest from a subsample of 3 cows per pen. Carcass traits were evaluated postharvest. There were no differences in live performance traits of ADG, DMI, or G:F among treatments. Additionally, no differences existed among treatments for most carcass traits. There was a trend ($P = 0.18$) for ZH cows to have larger LM areas than C cows. *Longissimus* lean color in the RH + ZH treatment tended ($P = 0.10$) to have higher L* values than the ZH group. Carcass fat had higher ($P < 0.05$) L* values in all treatments receiving β-AA supplementation. Expression of β_2-adrenergic receptor (AR) mRNA was not altered in the RH + ZH treatment during RH supplementation from d 24 to 51 of feeding. However, the expression of β_2-AR mRNA increased ($P < 0.05$) the last 23 d of feeding for the RH treatment and numerically increased ($P > 0.05$) in ZH cows during ZH supplementation. For all cows, expression of Type IIa myosin heavy chain (MHC-IIa) mRNA decreased ($P < 0.05$) after 24 d of feeding. Abundance of MHC-IIx mRNA increased ($P < 0.05$) for ZH and RH + ZH treatments the last 23 d of feeding during ZH supplementation. Although few significant differences were observed in

performance or carcass traits, mRNA expression indicated that β-AA supplementation elicited a cellular response in mature cows. Economically, implanting and feeding cull cows for 74 d, regardless of β-AA supplementation, added value by transiting cows from a "cull" cow to "white" cow market.

Key Words: realimentation, cull cows, ractopamine-HCl, zilpaterol-HCl, carcass and meat traits, β_2-adrenergic receptors

INTRODUCTION

Cows that are culled from herds because of problems associated with old age, reproductive inefficiency, lameness, disposition, or unsatisfactory performance can account for 15 to 20% of the income associated with cow-calf operations (Yager et al., 1980). Even though notable improvements have been made during the past decade, numerous problems are still associated with carcasses from cows. A recent study found that non-fed beef cows had lower HCW, smaller LM areas, leaner fat thicknesses, inferior muscling, yellower external fat color, and darker lean color than A-maturity, Select grade steers (Stelzleni et al., 2007). Feeding cull cows a high-energy ration prior to harvest can improve value, HCW, muscling, lean color, fat color, collagen solubility, and tenderness (Wooten et al., 1979; Miller et al., 1987; Boleman et al., 1996; Cranwell et al., 1996a, 1996b; Schnell et al., 1997; Sawyer et al., 2004). As a result of improved carcass and muscle characteristics, fed-cows, also known as white cows, almost always results in a premium price at harvest. Yet in order to obtain economic benefits of cull cow realimentation, producers must consider feed, yardage, and transportation costs, along with monitoring seasonal market prices of cows (Wright, 2005; Carter and Johnson, 2007).

Utilization of anabolic steroid implants in cow feeding programs can further improve ADG, feed efficiency, and lean meat yield (Matulis et al., 1987; Cranwell et al., 1996b; Funston et al., 2003). More recently, the use of commercially available β-adrenergic agonists (β-AA) have been evaluated in cull cow realimentation programs. In young cattle, β-AA have had profound effects on growth performance and carcass yields. However, in mature cows, effectiveness of β-AA is less pronounced. When ractopamine-HCl (RH) was added to cow feedlot rations at levels ranging from 100 to 300 mg·head^{-1}·d^{-1}, no differences were observed in performance or carcass characteristics compared to non-supplemented cows (Harborth, 2006; Carter et al., 2006; Dijkhuis et al., 2008; Holmer et al., 2009). Similarly, Neill et al. (2009) reported limited improvements in growth performance and carcass traits when cull cows were implanted and supplemented with zilpaterol-HCl (ZH). However, Strydom and Smith (2010) reported that supplementation with ZH improved feed efficiency, dressing

percent, and conformation scores in cull cows. Additionally, Gasch et al. (2010) reported that feeding 8.33 mg/kg of ZH to cull cows for 20 d increased ADG, carcass yield, LM area, and value of saleable yield compared to non-supplemented controls.

Several researchers have studied the influence of β-AA on expression of β-adrenergic receptor (β-AR) mRNA levels. There is a trend for RH supplementation during the last 28 d of finishing to increase expression of β_2-AR mRNA in heifers (Sissom et al., 2007) and steers (Winterholler et al., 2007). Gonzalez et al. (2008) reported similar findings in mature cows indicating that there is a trend for expression of β_2-AR mRNA in the *semimembranosus* to be increased after feeding RH for 28 d. Elevated levels of β_2-AR mRNA suggests that more β_2-AR are being translated, which leads to an increased density of receptors in skeletal muscle. Both reduced density of β-AR and deceased sensitivity of receptors to agonists have been reported in older humans and animals (Elfellah et al., 1989). Because ZH primarily elicits a response via β_2-AR, I hypothesized that feeding RH prior to ZH supplementation might up-regulate β_2-AR in mature cows and increase the effectiveness of ZH. Therefore, the objectives of this study were to explore the effects of feeding a single or sequence of β-AA to cull cows on feedlot performance, carcass traits, carcass composition, mRNA expression, and economics of production.

MATERIALS AND METHODS

Procedures involving cows were approved by the Kansas State University Institutional Animal Care and Use Committee.

Animals

Sixty crossbred, mature cows meeting established criteria (primarily of 'British' breeding, not pregnant, between 2 and 8 yr of age, between 454 and 590 kg, and a body condition score between 2 and 5) were procured from sale barns in western Kansas or were cull cows from the Agriculture Research Center in Hays, Kansas. One cow was removed from the study due to sickness and one because she had negative body weight gain.

Treatments

Cows were implanted in the right ear with Revalor®-200 (200 mg of trenbolone acetate and 20 mg estradiol; Intervet/Schering Plough Animal Health, Millsboro, DE) on d 0 and stratified by weight, body condition score, and ultrasound data into four treatments consisting of: 1) concentrate fed for 74 d (**C**); 2) concentrate fed for 49 d then supplemented with RH (Optalfexx®; Elanco, Greenfield, IN) for 25 d (**RH**); 3) concentrate fed for 51 d then supplemented with ZH (Zilmax®; Intervet/Schering Plough Animal Health, Millsboro, DE) for 20 d (**ZH**); 4), concentrate fed for 26 d then supplemented with RH for 25 d followed by ZH for 20 d (**RH + ZH**). All cows supplemented with ZH had a 3 d withdrawal prior to harvest. In the RH and RH + ZH treatments, RH was supplemented at level of 200 mg·head^{-1}·d^{-1}. In the ZH and RH + ZH treatments, ZH was supplemented at 8.33 mg/kg of feed.

Management

Body weights were recorded on all cows on d 0 of the feeding period. Cows were randomly allotted by treatment into pens of 5 resulting in 3 pens and 15 cows per treatment. Pen area was 220 m^2, bunk space was 6.0 m, and there was one water source per pen. Cows were fed a concentrate ration containing ground sorghum grain, sorghum silage and soybean meal (Table 3.1). On d 24, 51, and 72 of feeding, weights were recorded.

Muscle Biopsies

Longissimus muscle biopsy samples were collected on the left side between the 10th and 13th ribs on a subsample of 3 cows per pen on d 24, 51, and at harvest.   The same cows and relative locations were used for each sampling. Muscle biopsies were collected using procedures previously described (Dunn et al., 2003; Pampusch et al., 2003; Winterholler et al., 2007). Cows were restrained in a hydraulic chute, hair was clipped from the biopsy site, the site was thoroughly cleansed, and a local anesthetic (lidocaine HCl; 20 mg/mL; 8 mL per biopsy site) was administered. After approximately 5 to 8 min, non-response to a needle prick was used to determine if the biopsy site was properly numbed. The biopsy site was wiped with 70% ethanol and sterile gauze.

Tissue (approximately 1.5 g) was collected by making a 1-cm incision and using a 6-mm Bergstrom biopsy needle (Popper & Sons, Inc., New Hyde Park, NY). Biopsy samples were snap frozen in liquid nitrogen, stored on dry ice, and transported to the laboratory at Kansas State University for storage at -80°C until further analysis. Incision sites were closed with tissue adhesive (Vetbond; 3M, St. Paul, MN) and coated with a spray-on bandage (AluSpray; Neogen Corp., Lexington, KY). All biopsy sites were monitored for swelling 24 h after the biopsy procedure. There were no infections or problems resulting from the biopsies.

After hides had been removed at harvest and within 30 min postmortem, a sterile knife was used to collect LM samples from the same general location that live animal biopsies were obtained. Samples were snap frozen in liquid nitrogen, stored on dry ice, and transported to the laboratory at Kansas State University for storage at -80°C until RNA analysis.

Carcass Data

On d 74, cows were transported approximately 350 km to a commercial cow abattoir and humanely harvested. Hot carcass weights were recorded at harvest. After 72 h of chilling, carcasses were transported approximately 60 km to a fabrication facility, and all other carcass data were recorded. Carcass data collected included LM area; adjusted fat thickness; and percentage of KPH fat. After ribbing and a 30 min bloom time, marbling score (scale: 300 = Slight[00]; 400 = Small[00]; 500 = Modest[00]); skeletal, lean and final maturity (scale of 100 to 599: 200 = B[00]; 300 = C[00]; 400 = D[00]; 500 = E[00]); lean color (scale 1 to 7: 1 = black; 4 = moderately dark red; 7 = very light cherry red); subjective fat color (scale 1 to 5: 1 = bleached white; 3 = slightly yellow; 5 = canary yellow); and instrumental fat and LM color were recorded. Instrumental color readings were collected using a HunterLab MiniScan XE Plus Spectrophotometer (Model 45/0 LAV, 2.54-cm-diameter aperture, 10° standard observer, Illuminant A; Hunter Associates Laboratory, Reston, VA) to determine CIE L* (lightness), a* (redness), b* (yellowness). Three scans were taken and averaged to determine instrumental color values. A pH meter with glass probe electrode (Meat Probes, Inc., Topeka, KS) was used to determine LM pH.

9-10-11th Rib Cut Out

The procedure developed by Hankins and Howe (1946) was used to mark the 9-10-11th rib section on the left side of each carcass. At 4 d postmortem, beef rib primals (NAMP # 103) were collected from the fabrication line and the 9-10-11th rib section was removed from the primal rib. The weight of the 9-10-11th rib section was recorded. All soft tissue was separated from bone, and bone weights were recorded. From the posterior portion of the 9-10-11th LM section, a 2.54-cm steak was cut, closely trimmed of subcutaneous fat, weighed, and stored for LM compositional analysis. The steaks' compositional data were used as a measurement of the percentage of intramuscular fat in the LM, and compositional information was added back to the 9-10-11th rib compositional data. The remainder of the soft tissue was vacuum packaged and transported to the Kansas State University Meat Laboratory for grinding. The soft tissue was coarse ground through a 0.953-cm plate, mixed thoroughly, and fine ground through a 0.138-cm plate. A 250-g sample of the fine-ground tissue was frozen at -40°C until it was pulverized. Moisture and fat were determined on a pulverized sample using the CEM SMART (moisture, CEM SMART System 5; CEM Corporation, Matthews, NC) and SMART Trac (fat, CEM Smart Trac System Rapid Fat Analysis; CEM Corporation, Matthews, NC) systems (AOAC PVM – 1:2003; Keeton et al., 2003). Crude protein and ash were determined by AOAC methods 990.03 and 942.05.

RNA Isolation

A 100 mg frozen sample of LM biopsy tissue was placed in a polypropylene tube containing 1 mL of cold TRI Reagent (Applied Biosystems/Ambion, Foster City, CA) and homogenized for 1 min. Homogenates were transferred to sterile 1.5 mL microcentrifuge tubes and incubated at ambient temperature for 5 min. Chloroform (0.2 mL) was added to each microcentrifuge tube, vortexed for 30 s, and incubated at room temperature for 3 min before being centrifuged at 12,000 x g for 15 min. The aqueous phase was transferred to sterile 1.5 mL microcentrifuge tubes. Isopropyl alcohol was added in a 1:1 ratio, vortexed for 30 s, and incubated at ambient temperature for 10 min. To optimize RNA yields, samples were stored at -80°C for 10 min and then incubated overnight at -20°C. The following day, samples were thawed on ice for 15

min before being centrifuged at 12,000 x g for 10 min. The supernatant was removed, and the RNA pellet was washed by pipetting 1 mL of cold 75% ethanol over the pellet. Samples were lightly vortexed and centrifuged at 12,000 x g for 5 min. Supernatant was removed as before, and the RNA pellet was air-dried for 5 to 10 min. The RNA pellet was dissolved in 40 µL of nuclease-free water (QIAGEN Inc., Valencia, CA). Integrity and quantity of RNA was evaluated using the 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA) according to the manufacturer's instructions. If samples had a RNA integrity number less than 8, RNA was re-isolated from LM tissue. Samples were DNased using a commercially available kit (DNA-free; Applied Biosystems/Ambion, Austin, TX) to remove contaminating genomic DNA. One microgram of total RNA was reverse transcribed to produce the first strand of complimentary DNA using TaqMan reverse transcription reagents (Applied Biosystems/Ambion, Foster City, CA) and the protocol recommended by the manufacture. Random hexamers were used as primers in complimentary DNA synthesis.

Real-time Quantitative PCR

Real-time quantitative PCR was used to measure the expression of β_1, β_2, β_3, myosin heavy chain (MHC)-I, MHC-IIa, and MHC-IIx mRNA. Measurement of the relative quantity of cDNA was performed on 384-well plates using 2.5 µL of TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 0.45 µL of the appropriate forward and reverse primers (10µM), 0.01 µL of the appropriate TaqMan probe, 0.20 µL of sample, and 1.39 µL of nuclease-free water. Bovine primers, TaqMan probes, and accession numbers are presented in Table 3.2. Assays were performed using a 7900HT Fast Version 2.3 Sequence Detection System (Applied Biosystems, Foster City, CA) following the thermal cycling parameters recommended by the manufacturer (40 cycles of 15 s at 95ºC and 1 min at 60ºC). Relative expression was quantified by using the 2-ΔΔCt method. All sample values were normalized against ribosomal protein S9 and expressed in arbitrary units.

Statistical Analysis

The experimental design was a completely randomized design with sub-sampling. Data were analyzed as a one-way treatment structure using PROC GLM or PROC MIXED procedures in SAS (SAS Inst., Inc., Cary, NC). Pen was used as the experimental unit for feedlot performance and economic traits. Cow was used as the experimental unit for carcass characteristics and gene expression data. Satterthwaite adjustments were used for the degrees of freedom. Feedlot performance, economic traits, and carcass characteristic means were separated ($P < 0.05$) with the least significant difference procedures in SAS. Gene expression data were analyzed by appropriate contrast statements comparing treatments by slices of time.

RESULTS AND DISCUSSION

Performance Traits

There were no differences ($P > 0.05$) in initial weight, body condition score, or ultrasound data (Table 3.3) among treatments at the initiation of feeding. Least squares means for live animal performance traits are presented in Table 3.4. There were no differences ($P > 0.05$) in body weight gain, ADG, DMI or G:F among the treatments.

Other researchers have reported no change in performance traits with β-AA supplementation in mature cows. Feeding RH at 100, 200, or 300 mg·head^{-1}·d^{-1} during the last 28 to 35 d of feeding had no influence on cow performance (Harborth, 2006; Carter et al., 2006; Holmer et al., 2009). Neill et al. (2009) reported that implanted cows fed a concentrate ration with and without ZH supplementation had greater body weight gains the first 36 d of feeding compared to non-implanted cows. Implants may contribute to improved feedlot performance in mature cows to a greater extent than β-AA treatment. The lack of statistical differences in performance data in our study are likely due, in part, to high variability in performance within treatments, and unknown genetic and/or nutritional backgrounds among cows coming from different environments.

Carcass Traits

Least squares means for carcass traits and quality data are presented in Table 3.5. For most carcass characteristics, no differences ($P > 0.05$) existed among treatments. There was a trend ($P = 0.18$) for carcasses in the ZH treatment to have larger LM areas than carcasses in the C group. However, no differences ($P > 0.10$) were observed among treatments for marbling, LM percentage of intramuscular fat, fat thickness, yield grade, or maturity. Surprisingly though, the RH + ZH treatment had numerically the highest ($P = 0.30$) marbling score and percentage of intramuscular fat (5.0% vs. 4.4% for control). In young cattle, marbling can be negatively influenced by β-AA supplementation due to nutrients being repartitioned from adipose tissue towards skeletal muscle. In research by Neill et al. (2009), cows implanted and fed ZH for the final 30 d of finishing had improved LM area compared to cows fed only a concentrate ration, implanted and fed a concentrate ration, or non-implanted and supplemented with ZH. In my study, cows were only supplemented with ZH for 20 d, which may not have been long enough to see significant improvements in LM area.

There was a trend ($P = 0.10$) for LM lean L* values to be higher in carcasses from the RH + ZH treatment than in the ZH treatment. Holmer et al., (2009) reported that cows supplemented with RH for the final 35 d of finishing had a more youthful lean color than cows concentrate-fed without RH supplementation. They hypothesized that increased protein turnover caused a dilution effect of myoglobin and that increased protein denaturation occurred due to a more rapid decline in pH. I would postulate that the latter contributed to differences in lean color in our study because, although pH was not statistically different, cows in the sequential treatment had the lowest numerical pH (5.5) and cows from the ZH group had the highest numerical pH (5.7).

Subjective fat color scores were not different, but external fat was lighter (P < 0.05), as indicated by higher L* values, for all treatments receiving β-AA supplementation compared to controls. Other researchers have reported no differences in visual fat color as a result of β-AA supplementation (Harborth, 2006; Carter et al., 2006; Neill et al., 2009), or in contrast to our results, that β-AA supplementation decreased external fat L* values (Holmer et al., 2009). Generally, fat color improves with supplementation of a high-concentrate ration because less β-carotene from grass

is present in fat deposits (Dunne et al., 2009). French et al. (2000) suggested that whitening of fat is due to a dilution effect of new fat being deposited that has less carotene in it. I postulate that β-AA supplementation repartitioned fat towards lean development, which likely led to depletion of yellow fat and a greater dilution effect.

9-10-11th Rib Cutout

Least squares means for 9-10-11th rib section data are found in Table 3.6. No differences ($P > 0.05$) were found among treatments for rib section weight, bone weight or soft tissue weight. Additionally, there were no differences ($P > 0.05$) among treatments in regard to soft tissue composition of percent moisture, crude protein, total fat, or ash. Neill et al., (2009) reported that supplementing cows with a high-energy ration for 70 d, with or without implants or β-AA treatment, improved 9-10-11th rib cutout yields compared to grass feeding. However, similar to my results, no additional improvements to 9-10-11th rib cutout yields were observed when cows were implanted and supplemented with ZH.

mRNA Quantities

No differences were observed in β_1-AR mRNA expression due to β-AA supplementation (data not presented). In studies evaluating mRNA responses to β-AA in heifers (Sissom et al., 2007) and steers (Winterholler et al., 2007; Baxa et al, 2010), there was no change in β_1-AR mRNA abundance due to β-AA treatment. Baxa et al. (2010) went on to explain that no detectable changes of β_1-AR could be attributed to generally low expression, which results in differences hidden by sample-to-sample variation. I was unable to detect β_3-AR mRNA in LM biopsy samples collected from cows. This is not surprising because it is thought that β_3 receptors primarily reside in the few brown adipocytes remaining in and amongst the white adipose tissue of adult bovines (Sillence and Matthews, 1994).

Using contrasts statements, β_2-AR mRNA expression was evaluated among treatments and sample times. There were no differences among treatments for β_2-AR mRNA expression at d 24 of feeding. Contrary to my hypothesis, β_2-AR mRNA remained relatively constant throughout feeding for the RH + ZH treatment (Figure 3.1). Supplementation of RH from d 24 to 51 in the sequential treatment did not increase the

expression of β_2-AR mRNA. However, β_2-AR mRNA expression increased ($P \leq 0.05$) the last 23 d of feeding for the RH treatment and numerically increased ($P > 0.05$) for the ZH treatment concurrent with the supplementation of RH and ZH (Figure 3.1). Baxa et al. (2010) found that ZH supplementation increased β_2-AR mRNA expression in steers. Tendencies for increased β_2-AR mRNA expression were also observed in heifers (Sissom et al., 2007) and steers (Winterholler et al., 2007) due to RH treatment. Additionally, in mature cows fed RH there was a tendency for β_2-AR mRNA abundance to be increased (Gonzalez et al., 2008). However, all of these studies involved supplementation of β-AA during the last stages of finishing. I am uncertain as to why in my study RH increased β_2-AR mRNA during the later stages of feeding, but not from d 24 to 51 of realimentation. I postulate that there is potentially a time on feed and β-AA treatment interaction that needs to be further explored.

Increased β_2-AR mRNA expression in the RH and ZH treatments in my study suggests that a cellular response is being elicited by RH or ZH binding to receptors, which may lead to an up-regulation of β_2-AR density. However, elevated mRNA concentrations do not necessarily correspond to increased protein translation. Further analysis of protein quantification would need to be conducted to determine if up-regulation of β_2-AR density occurred. Additionally, mRNA expression of β-AR does not offer information regarding receptor sensitivity to agonists.

There was no change ($P > 0.05$) in MHC-I or MHC-IIa mRNA expression due to β-AA supplementation, but in all cows the expression of MHC-IIa mRNA decreased ($P < 0.05$) from d 24 to 51 of feeding (Figures 3.2 and 3.3). There was increased ($P < 0.05$) MHC-IIx mRNA expression from d 51 to harvest in cows fed ZH or a sequence of RH + ZH (Figure 3.4) during ZH supplementation the last 20 d of feeding.

In contrast to my study, Gonzalez et al. (2008) found that RH supplementation at 200 mg·head^{-1}·d^{-1} increased MHC-I mRNA compared to non-supplemented controls. Similar to my results, Baxa et al. (2010) reported no change in MHC-I mRNA abundance in the *semimembranosus* muscle due to ZH supplementation in steers. Conflicting results on the influence of β-AA on MHC-IIa have been published. Gonzalez et al. (2008) reported that supplementation of RH did not change the amount of detectible MHC-IIa mRNA. However, Baxa et al. (2010) found that ZH supplementation

had a tendency to decrease MHC-IIa expression. The decreased expression of MHC-IIa mRNA for all cows from d 24 to 51 of feeding could be due to a transition in muscle fiber types as a result of changing from a forage-based to concentrate ration. Vestergaard et al. (2000) suggested that forage-based-rations promote oxidative muscle metabolism instead of anaerobic metabolism. I postulate that throughout feeding of a high-energy ration, cow muscle fibers transitioned from oxidative muscle metabolism (Type I and IIa fibers) towards more glycolytic metabolism (Type IIx fibers).

In regard to MHC-IIx mRNA expression, similar results to my study were observed by Baxa et al. (2010) who reported that ZH supplementation increased the expression of MHC-IIx mRNA in steers. Rats selected for rapid postnatal growth have more Type IIx, fast-contracting muscle fibers (Sosnicki, 1987). In addition, double-muscled cattle and callipyge lambs typically have a greater proportion of fast glycolytic muscle fibers (Lefaucheur and Gerrard, 2000). Therefore, the increase in Type IIx fibers in my study for ZH and RH + ZH treatments suggests that cows supplemented with ZH should have greater potential for skeletal muscle growth which is consistent with a trend for larger LM area in cows receiving ZH supplementation.

Economic Impact

Economic analysis was conducted by comparing initial price of cows with harvest plant purchase price on a live animal basis. Feed costs were calculated by multiplying the cost of feed ingredients by pen feeding records. Implants were estimated at $5/pen, RH at $1.60·pen·d^{-1}, and ZH at $10.00·pen·d^{-1}. Transportation costs were estimated at $201.68/pen. No significant differences were found in initial value, cost of gain, end value, or net revenue among treatments (Table 3.7). However, cows increased in value from $54.50/cwt to $77.02/cwt over 74 d of feeding. In addition, cows from the ZH and RH + ZH treatments had numerically the highest net revenue, even with the added cost of ZH supplementation.

SUMMARY

Feeding implanted cull cows for 74 d, with or without β-AA supplementation, increased their value by approximately $23/cwt. Supplementation of cows with β-AA

during the last 20 to 25 d of feeding influenced mRNA expression similar to data reported in young cattle indicating that β-AA do elicit a cellular response in mature cows. However, implanting and supplementing mature cows with RH, ZH or a sequence of RH followed by ZH had few significant influences on performance traits or carcass characteristics, in part due to the large amount of variation that exists in mature cows acquired from different backgrounds.

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Table 3.1 Ingredient composition of experimental diets

Ingredient	% DM basis
Ground sorghum grain	76.95
Sorghum silage	20.04
Soybean meal (44%)	1.61
Minor/Supplement[1]	1.40

[1]Minor ingredients: urea, calcium, salt. For the ractopamine-HCl and ractopamine-HCl + zilpaterol-HCl treatments, ractopamine-HCl was fed at 200 mg·head^{-1}·d^{-1}; for the zilpaterol-HCl and ractopamine-HCl + zilpaterol-HCl treatments, zilpaterol-HCl was fed at 8.33 mg/kg of feed

Table 3.2 Sequences for bovine-specific PCR primers and TaqMan probes for β1-, β2-, β3-adrenergic receptors; types I, IIa, and IIx myosin heavy chain (MHC) mRNA; and ribosomal protein S9 (RPS9)

Item	Sequence (5' to 3')
β_1-Adrenergic receptor (accession No. AF188187)	
Forward	GTGGGACCGCTGGGAGTAT
Reverse	TGACACACAGGGTCTCAATGC
TaqMan probe	6FAM-CTCCTTCTTCTGCGAGCTCTGGACCTC-TAMRA
β_2-Adrenergic receptor (accession No. NM_174231)	
Forward	CAGCTCCAGAAGATCGACAAATC
Reverse	CTGCTCCACTTGACTGACGTTT
TaqMan probe	6FAM-AGGGCCGCTTCCATGCCC-TAMRA
β_3-Adrenergic receptor (accession No. X85961)	
Forward	AGGCAACCTGCTGGTAATCG
Reverse	GTCACGAACACGTTGGTCATG
TaqMan probe	6FAM-CCCGGACGCCGAGACTCCAG-TAMRA
MHC-I (accession # AB059400)	
Forward	CCCACTTCTCCCTGATCCACTAC
Reverse	TTGAGCGGGTCTTTCTTTTTCT
TaqMan probe	6FAM-CCGGCACGGTGGACTACAACATCATAG-TAMRA
MHC-IIa (accession # AB059399)	
Forward	CCCCGCCCCACATCTT
Reverse	TCTCCGGTGATCAGGATTGAC
TaqMan probe	6FAM-TCTCTGACAACGCCTATCAGTTCAT-TAMRA
MHC-IIx (accession # DT860044)	
Forward	GGCCACTTCTCCCTCATTC
Reverse	CCGCCACCGTCTCATTCA
TaqMan probe	6FAM-CGGGCACTGTGGACTACAACATTACT-TAMRA

Table 3.3 Least squares means of cow traits at the initiation of a 74 d feeding trial with or without β-adrenergic agonist supplementation

| | Treatment[1] | | | | | |
Item	C	RH	ZH	RH + ZH	SE	P-Value
Initial BW, kg	526.3	521.6	521.3	523.5	5.93	0.93
Initial BCS[2]	4.5	4.6	4.6	4.7	0.11	0.74
Initial Ultrasound						
Fat thickness, mm	3.5	3.4	3.0	3.7	0.26	0.40
Muscle depth, mm	39.3	40.7	40.2	40.7	0.86	0.66
Marbling score[3]	5.7	5.8	5.6	5.6	0.23	0.94

[1]Treatments: C = concentrate fed for 74 d; RH = concentrate fed for 49 d then supplemented with ractopamine-HCl for 25 d; ZH = concentrate fed for 51 d then supplemented with zilpaterol-HCl for 20 d; RH + ZH = concentrate fed for 26 d then supplemented with ractopamine-HCl for 25 d followed by zilpaterol-HCl for 20 d
[2]Body condition score: 1 = extremely thin, 9 = very obese
[3]Marbling score: 4.0 = Slight[00], 5.0 = Small[00], etc.

Table 3.4 Least squares means for feedlot performance of cows fed for a high-energy ration 74 d with or without β-adrenergic agonist supplementation

Item	C	RH	ZH	RH + ZH	SE	P-Value
			Treatment			
Final BW, kg	639	628	647	647	12.0	0.53
BW Gain, kg						
d 0 to 24	26.3	34.0	34.5	49.0	6.61	0.18
d 0 25 to 51	41.0	45.9	37.7	45.2	12.55	0.96
d 52 to 72	45.0	26.6	54.4	29.5	14.77	0.56
Total (d 0 to 72)	112.1	106.5	125.5	123.7	11.85	0.64
ADG, kg/d						
d 0 to 24	1.1	1.4	1.4	2.1	0.29	0.19
d 0 25 to 51	1.5	1.7	1.4	1.7	0.46	0.96
d 52 to 72	2.1	1.3	2.5	1.4	0.71	0.57
Total (d 0 to 72)	1.6	1.5	1.8	1.7	0.17	0.60
DMI, kg·head^{-1}·d^{-1}						
d 0 to 24	14.7	14.7	14.5	14.5	0.14	0.60
d 25 to 51	12.3	12.3	12.3	12.3	0.00	n/a
d 52 to 72	10.5	9.9	10.7	10.7	0.72	0.84
Total (d 0 to 74)	12.6	12.3	12.6	12.5	0.21	0.79
G:F						
d 0 to 24	0.07	0.09	0.10	0.14	0.020	0.17
d 0 25 to 51	0.12	0.14	0.12	0.14	0.038	0.97
d 52 to 72	0.18	0.12	0.22	0.12	0.056	0.52
Total (d 0 to 72)	0.12	0.12	0.14	0.14	0.012	0.58

[1]Treatments: C = concentrate fed for 74 d; RH = concentrate fed for 49 d then supplemented with ractopamine-HCl for 25 d; ZH = concentrate fed for 51 d then supplemented with zilpaterol-HCl for 20 d; RH + ZH = concentrate fed for 26 d then supplemented with ractopamine-HCl for 25 d followed by zilpaterol-HCl for 20 d

Table 3.5 Least squares means for carcass traits and quality characteristics of cows fed a high-energy ration for 74 d with or without β-adrenergic agonist supplementation

Trait	Treatment[1]				SE	P-value
	C	RH	ZH	RH + ZH		
HCW, kg	369.9	372.0	385.3	389.7	10.29	0.42
Dressing percentage, %	59.0	59.3	59.7	60.2	0.62	0.58
LM area, cm^2	83.6	85.8	93.6	91.4	3.22	0.18
Adj. fat thickness, cm	0.89	0.94	0.87	0.96	0.105	0.92
KPH fat, %	1.5	1.3	1.3	1.5	0.13	0.60
Yield grade	2.6	2.6	2.2	2.5	0.20	0.46
Marbling score[2]	383	357	373	403	17.3	0.30
LM intramuscular fat, %	4.4	4.1	4.0	5.0	0.38	0.26
Bone maturity[3]	508	495	553	515	30.3	0.56
Lean maturity[3]	330	331	337	275	41.2	0.68
Final maturity[3]	456	427	482	439	22.5	0.41
pH	5.6	5.6	5.7	5.5	0.06	0.55
Lean Color						
Objective Score[4]	5.4	5.6	5.4	4.4	0.42	0.27
L*	39.08	39.23	38.17	40.81	0.764	0.10
a*	27.95	28.30	27.53	28.87	1.228	0.88
b*	17.40	16.72	17.15	17.18	1.058	0.97
Fat Color						
Objective Score[5]	2.6	2.8	2.4	2.5	0.22	0.62
L*	73.74[b]	75.34[a]	75.96[a]	76.18[a]	0.574	0.02
a*	12.61	11.70	11.05	11.15	0.731	0.41
b*	22.27	23.49	23.72	22.03	1.073	0.58

[1]Treatments: C = concentrate fed for 74 d; RH = concentrate fed for 49 d then supplemented with ractopamine-HCl for 25 d; ZH = concentrate fed for 51 d then supplemented with zilpaterol-HCl for 20 d; RH + ZH = concentrate fed for 26 d then supplemented with ractopamine-HCl for 25 d followed by zilpaterol-HCl for 20 d
[2]Marbling score: 300 = Slight^{00}, 400 = Small^{00}, etc.
[3]Bone, Lean and Final maturity: 300 = C^{00}, 400 = D^{00}, 500 = E^{00}
[4]Objective Lean color: 1 = black, 4 = moderately dark red, 7 = very light cherry red
[5]Objective Fat color: 1 = bleached white, 3 = slightly yellow, 5 = canary yellow

Table 3.6 Least squares means for weights and percentages of the 9-10-11th rib section of cows fed a high-energy ration for 74 d with or without β-adrenergic agonist supplementation

| Trait | Treatment[1] | | | | SE | P-value |
	C	RH	ZH	RH + ZH		
9-10-11th rib, kg	6.7	6.8	7.0	7.1	0.21	0.58
Bone, kg	1.2	1.2	1.3	1.2	0.04	0.61
Soft tissue, kg	5.5	5.6	5.8	6.0	0.19	0.42
Moisture, %	51.4	51.8	53.3	51.9	1.21	0.67
CP, %	15.7	16.2	16.7	16.3	0.37	0.34
Total fat, %	30.9	29.9	28.4	29.9	1.53	0.71
Ash, %	0.80	0.78	0.83	0.79	0.017	0.30

[1]Treatments: C = concentrate fed for 74 d; RH = concentrate fed for 49 d then supplemented with ractopamine-HCl for 25 d; ZH = concentrate fed for 51 d then supplemented with zilpaterol-HCl for 20 d; RH + ZH = concentrate fed for 26 d then supplemented with ractopamine-HCl for 25 d followed by zilpaterol-HCl for 20 d

Figure 3.1 β_2-AR mRNA expression in bovine LM collected from cows fed a high-energy ration for 74 d with or without β-adrenergic agonist supplementation Treatments: C = concentrate fed for 74 d; RH = concentrate fed for 49 d then supplemented with ractopamine-HCl for 25 d; ZH = concentrate fed for 51 d then supplemented with zilpaterol-HCl for 20 d; RH + ZH = concentrate fed for 26 d then supplemented with ractopamine-HCl for 25 d followed by zilpaterol-HCl for 20 d
*Increased ($P < 0.05$) expression from d 51 to harvest for RH treatment
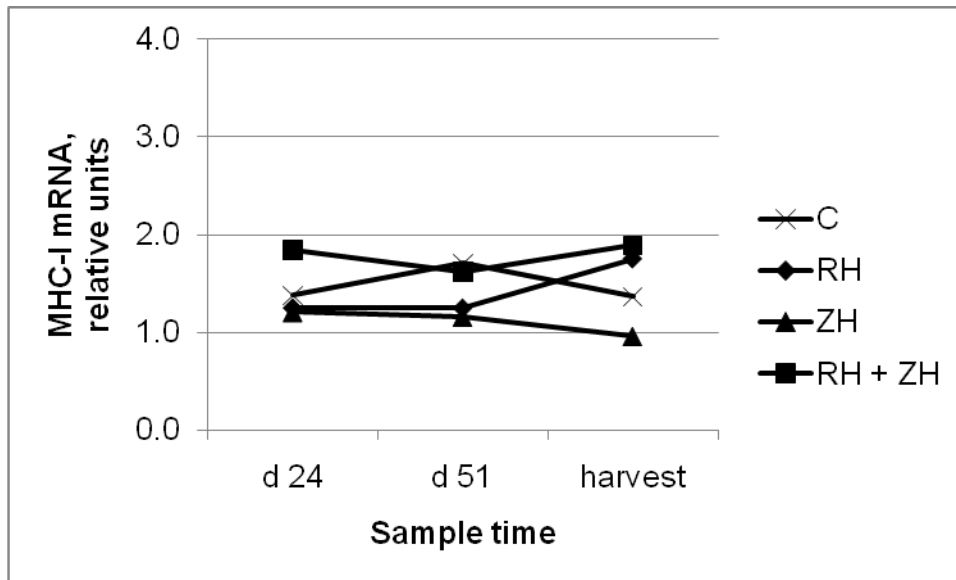
Figure 3.2 Type I myosin heavy chain (MHC-I) expression in bovine LM collected from cows fed a high-energy ration for 74 d with or without β-adrenergic agonist supplementation

Treatments: C = concentrate fed for 74 d; RH = concentrate fed for 49 d then supplemented with ractopamine-HCl for 25 d; ZH = concentrate fed for 51 d then supplemented with zilpaterol-HCl for 20 d; RH + ZH = concentrate fed for 26 d then supplemented with ractopamine-HCl for 25 d followed by zilpaterol-HCl for 20 d
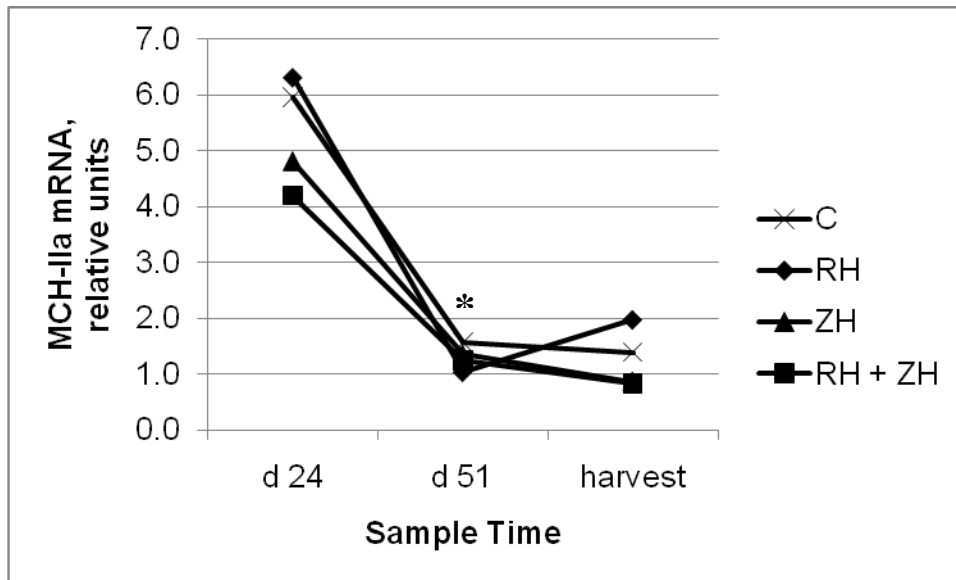
Figure 3.3 Type IIa myosin heavy chain (MHC-IIa) expression in bovine LM collected from cows fed a high-energy ration for 74 d with or without β-adrenergic agonist supplementation

Treatments: C = concentrate fed for 74 d; RH = concentrate fed for 49 d then supplemented with ractopamine-HCl for 25 d; ZH = concentrate fed for 51 d then supplemented with zilpaterol-HCl for 20 d; RH + ZH = concentrate fed for 26 d then supplemented with ractopamine-HCl for 25 d followed by zilpaterol-HCl for 20 d
*Decreased (P <0.05) expression from d 24 to 51 for all treatments
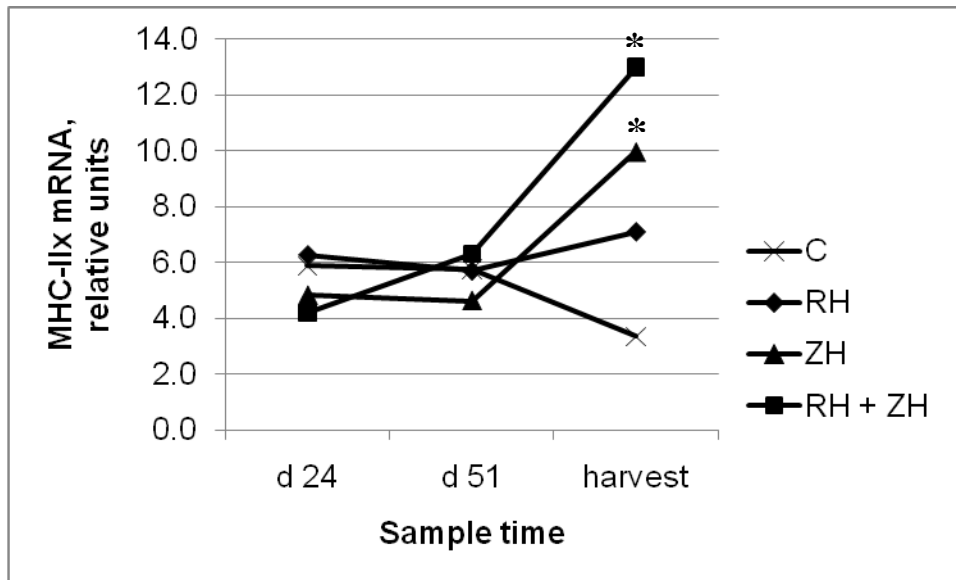
Figure 3.4 Type IIx myosin heavy chain (MHC-IIx) mRNA expression in bovine LM collected from cows fed a high-energy ration for 74 d with or without β-adrenergic agonist supplementation

Treatments: C = concentrate fed for 74 d; RH = concentrate fed for 49 d then supplemented with ractopamine-HCl for 25 d; ZH = concentrate fed for 51 d then supplemented with zilpaterol-HCl for 20 d; RH + ZH = concentrate fed for 26 d then supplemented with ractopamine-HCl for 25 d followed by zilpaterol-HCl for 20 d

*Increased ($P < 0.05$) expression of MHC-IIx from d 51 to harvest for ZH and RH + ZH treatments

Table 3.7 Least squares means for economic values related to cows fed a high-energy ration for 74 d with or without β-adrenergic agonist supplementation

Trait	Treatment[1]				SE	P-value
	C	RH	ZH	RH + ZH		
Initial value/head	$616.90	$618.24	$622.06	$622.03	$8.08	0.95
Cost/kg of gain	$1.82	$1.86	$1.74	$1.59	$0.20	0.80
End value/head	$1,056.71	$1,066.66	$1,098.31	$1,098.82	$19.76	0.37
Net revenue/head	$191.20	$199.43	$226.03	$226.44	$18.86	0.47

[1]Treatments: C = concentrate fed for 74 d; RH = concentrate fed for 49 d then supplemented with ractopamine-HCl for 25 d; ZH = concentrate fed for 51 d then supplemented with zilpaterol-HCl for 20 d; RH + ZH = concentrate fed for 26 d then supplemented with ractopamine-HCl for 25 d followed by zilpaterol-HCl for 20 d
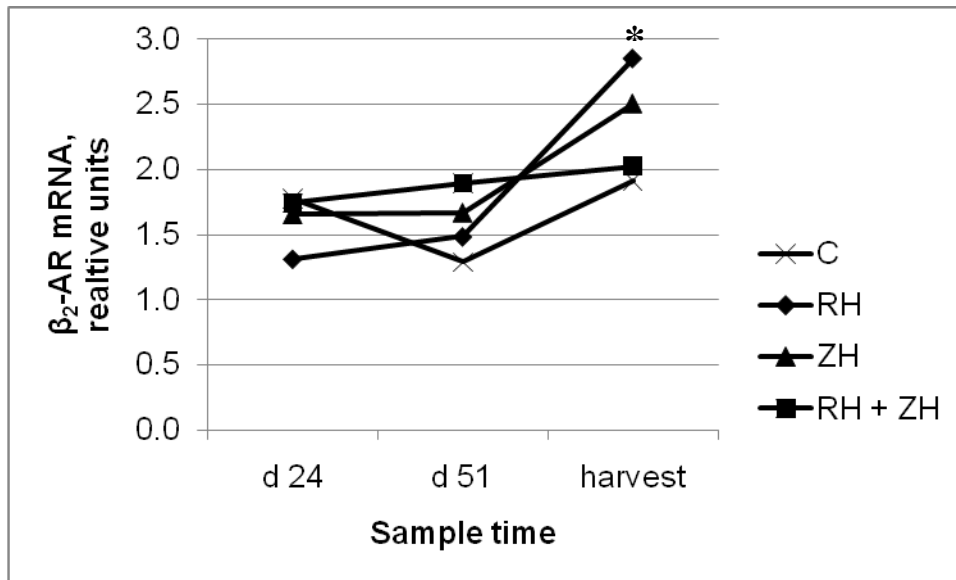
Chapter 4 - Effects of sequential feeding of β-adrenergic agonists on cull cow muscle biochemical and meat sensory characteristics

ABSTRACT

The objectives of this study were to investigate the effects of feeding a single or sequence of β-adrenergic agonists (β-AA) to cull cows on meat color and sensory characteristics as well as the ability of calcium lactate enhancement to improve muscle sensory profiles of β-AA-supplemented cows. Sixty cull cows were implanted with Revalor®-200 and assigned to 1 of 4 treatments: C = fed a concentrate ration; RH = supplemented with ractopmaine-HCl (RH) for the last 25 d; ZH = supplemented with zilpaterol-HCl (ZH) for the last 20 d; RH + ZH = supplemented with RH for 25 d followed by ZH for the last 20 d. All cows were fed the concentrate ration for 74 d. At 4 d postmortem, the primal rib, shoulder clod, and tenderloin from the left side of carcasses were retrieved. Steaks from the 6-7-8th rib section of the LM were used for desmin degradation analysis (aged for 10 or 21 d); collagen quantification (aged for 21 d) and Warner Bratzler shear force (WBSF, aged for 21 d) measurement. The 12th rib section of the LM was enhanced with a 0.1 M calcium lactate solution at 7 d postmortem, aged for an additional 7 d, and evaluated for WBSF and sensory panel ratings. *Psoas major* steaks (aged for 21 d) were evaluated for WBSF and sensory panel ratings, and WBSF measurement was performed on *infraspinatus* steaks (aged for 14 d). The remainder of the shoulder clod was used to produce 85/15 ground beef that was PVC overwrapped and evaluated for color shelf-life. *Psoas major* steaks from the sequential β-AA treatment (RH + ZH) were rated as more tender ($P < 0.05$) by sensory panelists than steaks from all other treatments. *Infraspinatus* steaks from the cows supplemented with β-AA had improved ($P < 0.05$) WBSF values. Non-enhanced LM steaks from cows fed ZH tended ($P = 0.12$) to have higher WBSF values and decreased ($P < 0.0.0001$) postmortem proteolysis at 10 and 21 d postmortem than steaks from cows not fed β-AA or fed RH only. The percentage of soluble collagen was increased ($P < 0.05$) by ZH supplementation compared to RH and control treatments. Enhancement of the LM with

a 0.1 M calcium lactate solution alleviated differences in WBSF values between β-AA supplemented and control cows. No differences were observed in ground beef color stability among treatments. Sequential feeding of RH followed by ZH yielded no detrimental effects on color or sensory properties of meat from mature cows compared to feeding a single β-AA.

Key Words: realimentation, cull cows, beta-adrenergic agonists, tenderness, ground beef color

INTRODUCTION

Cows culled from cow-calf, seedstock, or dairy operations account for 17 to 19% of all cattle harvested in the U.S. each year equaling approximately 80,000 metric tons of boneless beef (Woerner, 2010). A majority of meat from market cows is utilized for ground beef production. However, the most recent National Market Cow and Bull Audit (NCBA, 2007) revealed that all plants included in the audit produced rib and loin cuts from some market cows and bull carcasses, and an increased number of plants were fabricating merchandisable cuts from all primals. Even though notable improvements have been made during the past decade, numerous problems are associated with meat from mature cows. A recent study found that non-fed beef cows had lower HCW, smaller LM areas, less fat thickness, inferior muscling, yellower external fat color, darker lean color, and inferior sensory ratings for tenderness and off-flavor compared to A-maturity, Select grade steers (Stelzleni et al., 2007).

Realimentation of cows by feeding a high-energy ration prior to harvest can improve HCW, muscling, lean color, fat color, collagen solubility, tenderness, and flavor profiles (Wooten et al., 1979; Miller et al., 1987; Boleman et al., 1996; Schnell et al., 1997; Sawyer et al., 2004; Patten et al., 2008). In an attempt to efficiently improve value of mature cows, the commercially available β-adrenergic agonists (β-AA) ractopamine-HCl (RH) and zilpaterol-HCl (ZH) have been evaluated in cull cow realimentation programs. In young cattle, β-AA have profound effects on growth performance and carcass yields. However, in mature cows, effectiveness of β-AA has been limited (Harborth, 2006; Carter et al., 2006; Dijkhuis et al., 2008; Holmer et al., 2009a; Neill et al., 2009). Several researchers have studied the influence of β-AA on β-adrenergic receptor (β-AR) mRNA levels. There is a trend for RH supplementation during the last 28 d of finishing to increase β_2-AR mRNA levels in heifers (Sissom et al., 2007), steers (Winterholler et al., 2007), and mature cows (Gonzalez et al., 2008). I hypothesized that feeding RH prior to ZH supplementation might up-regulate β_2-AR and increase the effectiveness of ZH.

Although, supplementation with β-AA can improve lean meat yields by increasing protein synthesis and decreasing protein degradation (Mersmann, 1998), meat sensory

characteristics can be negatively affected. Mixed data have been published regarding the influence of β-AA on beef color shelf-life. Neill et al. (2009) and Gonzalez et al. (2009) reported that by d 5 of retail display, β-AA supplementation had detrimental effects on color shelf-life. Other studies have reported that in both the *semimembranosus* and LM, supplementation of young feedlot cattle with ZH for 20 or 30 d resulted in similar, if not better, color shelf-life than control treatments (Gunderson et al., 2009; Hilton et al., 2009).

In regard to tenderness, it is widely accepted that high calpastatin activity is responsible for interference of normal postmortem tenderization in β-AA supplemented cattle (Wheeler and Koohmaraie, 1992). In young cattle, it is estimated that there is a 1.1 to 1.7 kg increase in Warner Bratzler shear force (WBSF) values in 7-d-postmortem-aged LM steaks; however, this is diminished by 21-d-postmortem to a 0.27 to 1.4 kg increase (Claus et al., 2010; Hilton et al., 2009; Kellermeier et al., 2009). Holmer et al. (2009b) reported that feeding RH to cows had no adverse affects on sensory proprieties of 10 muscles. However, Hutchison (2007) reported that sensory panelists rated ZH supplemented cows as tougher and to contain more connective tissue than non-supplemented cows. In addition, LM steaks from ZH treated cows had the highest WBSF values.

To overcome reduced postmortem muscle proteolysis, the use of calcium salts has been evaluated.   Lawrence et al. (2003) demonstrated that calcium salts, such as calcium chloride, calcium lactate, and calcium ascorbate, increase tenderness by activating calcium-dependent enzymes. Therefore, the objectives of this study were to explore the effects of feeding a single or sequence of β-AA to cull cows on meat color and sensory characteristics as well as the ability of calcium lactate enhancement to improve sensory profiles of meat from β-AA-supplemented cows.

MATERIALS AND METHODS

Procedures involving cows were approved by the Kansas State University Institutional Animal Care and Use Committee. Procedures involving human subjects were approved by the Kansas State University Institutional Review Board.

Animals

Sixty crossbred, mature cows meeting established criteria (primarily of 'British' breeding, not pregnant, between 2 and 8 yr of age, between 454 and 590 kg, and a body condition score between 2 and 5) were procured from sale barns in western Kansas or were cull cows from the Agriculture Research Center in Hays, Kansas. One cow was removed from the study due to sickness and one because she had negative body weight gain.

Treatments

Cows were implanted in the right ear with Revalor-200 (200 mg of trenbolone acetate and 20 mg estradiol; Intervet/Schering Plough Animal Health, Millsboro, DE) on d 0 and stratified by weight, body condition score, and ultrasound data and assigned randomly into four treatments consisting of: 1) concentrate fed for 74 d (**C**); 2) concentrate fed for 49 d then supplemented with RH (Optalfexx, Elanco, Greenfield, IN) for 25 d (**RH**); 3) concentrate fed for 51 d then supplemented with ZH (Zilmax, Intervet/Schering Plough Animal Health, Millsboro, DE) for 20 d (**ZH**); 4), concentrate fed for 26 d then supplemented with RH for 25 d followed by ZH for 20 d (**RH + ZH**). All cows supplemented with ZH had a 3 d withdrawal prior to harvest. Cow performance, carcass data and mRNA responses are reported in Chapter 3.

Subprimal Fabrication/Processing

At 4 d postmortem, the primal rib (NAMP # 103), shoulder clod (NAMP # 114), and tenderloin (NAMP # 189) from the left side of carcasses were retrieved from the fabrication line at the processing facility. The 9-10-11th rib section was separated from the rest of the primal rib to estimate carcass composition (Hankins and Howe, 1946) and results are reported in Chapter 3. Bone was removed from the 6-7-8th rib section of the LM and subcutaneous fat closely trimmed. Three 1.91-cm steaks were cut, starting from the anterior end, and vacuum packaged for collagen and desmin degradation analyses. One steak for desmin analysis was frozen at -40°C after 10 d of postmortem aging, the second desmin steak and a collagen steak were frozen at -40°C after 21 d postmortem aging. Two 2.54-cm steaks were cut from the posterior portion of the 6-7-

8th rib LM were aged for 21 d at 2°C before base Warner Bratzler shear force (WBSF) measurements were evaluated. The 12th rib section of the LM was enhanced with a calcium lactate solution at 7 d postmortem. After enhancement, 2.54-cm steaks were cut and vacuum packaged for WBSF and sensory evaluation. Enhanced steaks were aged for an additional 7 d after enhancement. Steaks used for WBSF measurements were not frozen, but sensory steaks were frozen at -40°C until sensory panels could be conducted.

Tenderloins were transported to Kansas State University and subjected to 21 d of postmortem aging before two 2.54-cm steaks were cut for WBSF and two 2.54-cm steaks cut for sensory evaluation. Steaks for WBSF were not frozen, but sensory steaks were frozen at -40°C until sensory panels could be conducted. The *infraspinatus* muscle was removed from shoulder clods and two 2.54-cm steaks were cut and subjected to 14 d of postmortem aging prior to WBSF evaluation.

The remainder of the shoulder clod was closely trimmed of fat and then approximately 85 % lean, 15 % fat ground beef was produced by grinding product through a 0.953-cm plate, mixing thoroughly, and fine grinding through a 0.138-cm plate. Ground beef (~0.98 kg) was packaged in polyvinyl chloride (PVC) on 20.32 cm x 14.61 cm x 1.74 cm foam trays (2S, Cryovac Sealed Air, Duncan, SC) and overwrapped with oxygen permeable film (MAPAC M film, 23,250 cc/m2/24h, 72 gauge, Resinite Packaging Films, Borden, Inc., North Andover, MA) for retail color display.

Enhancement

Longissimus roasts from the 12th rib portion of the rib primal were used for enhancement. Roasts were injected (Model Imax 420; Wolftec, Inc., Werther, Germany) with a 0.1 M calcium lactate (PURAC America, Inc., Lincolnshire, IL) brine to a target 11% pump. After enhancement, roasts were allowed to sit for 1 h before 2.54-cm steaks were cut and aged for WBSF and sensory evaluation.

Cooking of steaks

Steaks for WBSF and sensory analysis were cooked in a forced-air convection oven (Blodgett, model DFG-102 CH3, G.S. Blodgett Co., Burlington, VT) set at 163°C. Steaks were turned at an internal temperature of 40°C and cooked to an endpoint

internal temperature of 70°C, as monitored by copper-constantan thermocouples in the approximate geometric center of each steak.

Warner-Bratzler shear force (WBSF)

On 14 d postmortem, *infraspinatus* and enhanced LM steaks were cooked for WBSF determination. For the *infraspinatus* muscle, the connective tissue strip was avoided when coring samples for WBSF measurement. On 21 d postmortem, base tenderness LM steaks and *psoas major* steaks were cooked for WBSF determination. All steaks were cooked according to the procedures described above, cooled to room temperature, and stored at 2°C ± 2°C overnight. Eight 1.27-cm cores were removed parallel to the muscle fibers using a 1.27-cm corer (G-R Manufacturing Co., Manhattan, KS) attached to an electric drill (Craftsman 3/8" Electric Drill, Sears, Hoffman Estates, IL). Cores were sheared perpendicular to the muscle fibers using a WBS Testing Machine (G-R Elec. Mfg. Co., Manhattan, KS). The machine was set to zero and the shear blade was cleaned before each shear. The shear force of each core was recorded in kg.

Sensory Analysis

Enhanced LM steaks, aged to 14 d postmortem, and *psoas major* steaks, aged to 21 d, were subjected to sensory panel evaluation. Panelists (n = 8) were trained according to AMSA guidelines (1995) for evaluation of steaks. Steaks were thawed overnight (2 ± 2°C), cooked according to procedures stated above, sliced into 2.54 cm x 1.27 cm x 1.27 cm samples, and served warm to panelists. Samples were kept warm in enamel, double-broiler pans with warm water in the bottom portion of the pan. Panelists evaluated samples in duplicate for myofibrillar tenderness, juiciness, beef flavor intensity, amount of connective tissue, overall tenderness, and off-flavor intensity using an eight-point scale. The scale used for myofibrillar and overall tenderness was: 1 = extremely tough, 2 = very tough, 3 = moderately tough, 4 = slightly tough, 5 = slightly tender, 6 = moderately tender, 7 = very tender, and 8 = extremely tender. For juiciness, the scale was: 1 = extremely dry, 2 = very dry, 3 = moderately dry, 4 = slightly dry, 5 = slightly juicy, 6 = moderately juicy, 7 = very juicy, and 8 = extremely juicy. The scale used for beef flavor was: 1 = extremely bland, 2 = very bland, 3 = moderately bland, 4 =

slightly bland, 5 = slightly intense, 6 = moderately intense, 7 = very intense, and 8 = extremely intense. The scale used for connective tissue and off flavor intensity was: 1 = abundant, 2 = moderately abundant, 3 = slightly abundant, 4 = moderate, 5 = slight, 6 = traces, 7 = practically none, and 8 = none. Scores were given to the nearest half-point increment.

Immunoblotting Procedures

Desmin degradation was used as a measure of postmortem proteolysis. Extraction, electrophoresis, Western blotting, and quantification of desmin was measured on LM samples aged for d 10 and 21 postmortem. Desmin degradation analysis was carried out in conjunction with scientists at the Roman L. Hruska U.S. Meat Animal Research Center in Clay Center, NE according to procedures outlined by Wheeler and Koohmaraie (1999) and Wheeler et al. (2002). Reagents were made with Sigma chemicals (Sigma-Aldrich, St. Louis, MO) and reagents used for electrophoresis were obtained from Bio-Rad Laboratories (Hercules, CA) unless otherwise stated. Raw LM samples were pulverized in liquid nitrogen. Muscle extracts were done by homogenizing 1 g of muscle in 10 mL of 50 mM Tris, 10mM EDTA, pH 8.3, for 20 s using a polytron on speed setting 24 (Brinkmann Instruments, Westbury, NY). Sample homogenate (0.5 mL) and 0.5 mL 2× treatment buffer (0.125 M tris, 4% sodiumdodecyl sulfate (SDS), and 20% glycerol; pH 6.8) were vortexed and incubated in a 50°C waterbath for 20 min, mixed with repeat pipetting, and heated for an additional 5 min. Samples were then centrifuged (Eppendorf 5414 C, Eppendorf AG, Hamburg, Germany) at 16,000 x g for 20 min to pellet insoluble material.

Protein concentration was determined using the micro-BCA protein assay (Pierce, Rockford, IL) with a 1:5 dilution of supernatant and 1× treatment buffer in triplicate. BCA reagent (200 µL) was added to each sample, incubated at 37°C for 30 min, and read at 562 nm (SPECTRAmax Plus 384, Molecular Devices Corp., Sunnyvale, CA). Samples were diluted to 3 mg/mL using protein denaturing buffer (PDB) containing 2× treatment buffer (listed above), 0% mercaptoethanol (MCE), and 0.8% bromophenol blue. Samples were vortexed, heated in a waterbath at 50°C for 10 min, and frozen until used for electrophoresis.

Desmin was separated on 10% gels (1.5 M tris, pH 8.8; 30% acrylamide (37.5:1 acrylamide to bisacrylamide); 10% SDS; 10% ammonium persulfate (APS); and TEMED) with 4% stacker (0.5 M tris, pH 6.8; 30% acrylamide (37.5:1); 10% SDS; 10% APS; and TEMED) in buffer containing 0.25 M tris and 1.92 M glycine. Samples were loaded at 15 μg of protein per lane and 0 h standards were loaded in triplicate at 18 μg of protein per lane. Discontinuous gels were run at 200 V for approximately 45 min. Gels were transferred to Hybond-P PVDF membranes (Amersham Biosciences, Piscataway, NJ) for 1 h at 200 mA in buffer containing 25 mM tris, 193 mM glycine, and 10% methanol. Membranes were blocked with 2.5% sheep serum in tris-buffered saline (20 mM tris, 137 mM NaCl, and 5 mM KCl), pH 7.4, containing 0.05% Tween-20 (TTBS) for 1 h. Membranes were incubated with gentle shaking at room temperature for 1 h with primary antibody as follows: monoclonal anti-desmin 1:300 (clone D3; developed by D. A. Fischman and obtained from the Developmental Studies Hybridomal Bank). Membranes were washed once for 15 min and twice for 5 min using TTBS. Bound primary antibodies were labeled (1 h at room temperature) with Immunopure goat anti-mouse IgG horseradish peroxidase conjugated secondary antibodies diluted 1:10,000 (Pierce, Rockford, IL). Membranes were washed once for 15 min and 4 times for 5 min with TTBS. Detection of antibody binding was done by incubating the membranes for 5 min using the SuperSignal West Dura Extended Duration Substrate (Pierce, Rockford, IL) and exposing the membrane for 5 min with a ChemiImager 4000 digital imaging analysis system (Alpha Innotech, San Leandro, CA). Protein bands were quantified using the ChemiImager 4000 digital imaging analysis system. Each blot contained three lanes of at-death (0 h postmortem) beef longissimus muscle samples that were averaged as a reference standard. The extent of desmin degradation was determined by expressing the density of protein bands of treatments as a percentage of degradation relative to that of the reference standard within each blot.

Collagen

Collagen assays were performed on a subsample (9 per treatment) of LM samples. Pulverized LM samples were stored at -80°C until collagen assays could be performed. Heat-labile collagen was extracted from duplicate 3 g samples by heating

for 80 min at 70°C in ¼ strength Ringer's solution (Hill, 1966). Following centrifugation at 5200 x g (Beckman Model J2-21, Beckman Coulter, Inc., Brea, CA), supernatants were separated from residues by filtration through Whatman # 1 filter paper. Ringer's (8 mL of ¼ strength) was added to resides, vortexed, and centrifuged again at 5200 x g. Supernatants were filtered to allow separation of soluble and insoluble fractions. Supernatants and residues were then hydrolyzed (autoclaved at 121°C under 1.2 kg/cm^2) in 6N HCl for 12 h. After samples cooled to room temperature, 1 g of carbon decolorizing agent was added to both soluble and insoluble collagen fractions and samples were vortexed. Samples were filtered and soluble fractions (supernatants) were diluted to 250 mL and insoluble fractions (residues) were diluted to 500 mL. Hydroxproline content was determined in duplicate for both fractions by spectrophotometric methods (Bergman and Loxley, 1963). One milliliter of diluted sample was combined with 2 mL of isopropyl alcohol and vortexed. Then, one milliliter of oxidant solution was added and samples were allowed to react for 4 min. Erhlich's reagent (4 mL) was added. Samples were vortexed and placed in a 60°C waterbath for 25 min. After cooling for 5 min, absorbance was read at 558 mn (Hitachi Model U-2010 UV/Vis Spectrophotometer; Hitachi High Technologies America, Inc., Naperville, IL). Concentrations of hydroxyproline were determined by using plotted values of known concentrations. Collagen content was calculated by multiplying the hydroxyproline content of the insoluble fraction by 7.25 and the insoluble fraction by 7.52 (Cross et al., 1973). Proportion of soluble collagen was calculated by dividing soluble collagen by the sum of the insoluble and soluble fractions.

Display Cases

Ground beef packages were displayed in open top display cases (Unit model DMF8, Tyler Refrigeration Corp., Niles, MI) under continuous fluorescent lighting (2153 lux, 3000 K and CRI = 85, Bulb model F32T8/ADV830/Alto, Philips, Bloomfield, NJ) for 3 d at 2 ± 3°C. Cases defrosted twice daily at 12 h intervals. Packages were rotated twice daily in order to maintain random sample placement and to account for temperature and lighting variation within display cases.

Color Measurements

Trained visual color panelists (n = 8), who had passed Fransworth-Munsell 100 Hue Test (MacBeth; Newburgh, NY), evaluated initial color on d 0 of display and display color and surface discoloration twice per d on d 0 to 3 of display. Initial color was evaluated to the nearest 0.5 point using the following scale: 1 = Bleached, pale red, 2 = Slightly cherry red, 3 = Moderately light cherry red, 4 = Cherry red, 5 = Slightly dark red, 6 = Moderately dark red, 7 = Dark red, 8 = Very dark red. The scale used by panelists to evaluate display color was: 1 = Very bright red, 2 = Bright red, 3 = Dull red, 4 = Slightly dark red, 5 = Slightly dark red or reddish tan, 6 = Moderately dark red to tannish red, 7 = Tan to brown. Display color was reported to the nearest 0.5 point. A score of 5.5 was considered borderline acceptable. Surface discoloration was evaluated to the nearest whole number using the following scale: 1 = None (0%), 2 = Slight (1-19%), 3 = Small (20-39%), 4 = Modest (40-59%), 5 = Moderate (60-79%), 6 = Extensive (80-99%), 7 = Total (100%).

Instrumental color was measured using a HunterLab MiniScan XE Plus Spectrophotometer (Model 45/0 LAV, 2.54-cm-diameter aperture, 10° standard observer; Hunter Associates Laboratory, Inc., Reston, VA) twice a d for the 3-d display period. Three scans were taken on each ground beef package and averaged for CIE L* (lightness), a* (redness), b* (yellowness) values. Hue angle was calculated using the $\tan^{-1} b^*/a^*$ and saturation index was calculated using $(a^{*2} + b^{*2})^{1/2}$.

Thiobarbituric acid reactive substances (TBARS) analysis

A modified procedure of Witte et al. (1970) was used for extraction and quantification of thiobarbituric acid reactive substances (TBARS) as a measurement of lipid oxidation. A representative sample of initial and end of display ground beef (approximately 100 g) was frozen in liquid nitrogen and pulverized using a tabletop blender (model 33BL79; Waring Products, New Hartford, CT). Pulverized samples (10 g) were blended with 10 mL of water and 15 mL of perchloric acid for 30 s. Samples were filtered (Cat. No. 1002, 125mm dia; Whatman International Ltd., Maidstone, England), and 5 mL of thiobarbituric acid solution was added to the filtrate. After samples were allowed to react at approximately 27°C for 18 h, absorbance at 529.5 nm

was read on a Spectophic 21 spectrophotometer (Bausch & Lomb, Rochester, NY). Control solutions of known concentrations of malonaldehyde were plotted to calculate TBA`RS concentration. The TBARS are reported at mg malonaldehyde per 1 g meat sample.

Statistical Analysis

Data were analyzed as a completely randomized design with a one-way treatment structure using the PROC MIXED procedure in SAS (SAS Inst., Inc., Cary, NC). Cow was used as the experimental unit. Satterthwaite adjustments were used for the degrees of freedom. Treatment means for sensory characteristics, desmin degradation, and collagen were separated ($P < 0.05$) with the least significant difference procedures in SAS. For ground beef color data, day was used as a repeated measure and the effects of treatment, day and the treatment by day interaction were analyzed and means separated ($P < 0.05$) with the least significant difference procedures in SAS.

RESULTS AND DISCUSSION

Psoas major and infraspinatus sensory characteristics

Least squares means for sensory characteristics and WBSF data are reported in Table 4.1. *Infraspinatus* steaks from cows supplemented with β-AA and subjected to 14 d of aging had lower (*P < 0.05*) WBSF values than steaks from control cows. Even though collagen assays were not performed on *infraspinatus* samples, I postulate that cows supplemented with β-AA had increased protein synthesis promoting the synthesis of new collagen. It is thought that newly synthesized collagen has fewer stabilized cross-links and should be more heat-labile (Etherington, 1981). Additionally, there may have been increased muscle fiber diameter from increased muscle hypertrophy in the *infraspinatus*, therefore, diluting the concentration of collagen. Gonzalez et al. (2008) reported no change in *infraspinatus* fiber diameter in cows supplemented with RH. However, when Kellermeier et al. (2009) analyzed LM fiber diameter in ZH supplemented steers, an increase of 5.18% was observed. Therefore, improvements in tenderness in the *infraspinatus* muscle of cows were likely due to improved collagen solubility, not myofibrillar tenderness differences.

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Sensory characteristics were evaluated on *psoas major* steaks after 21 d of postmortem aging. There were no differences (*P > 0.05*) among treatments for *psoas major* sensory panel ratings for juiciness, beef flavor intensity, connective tissue amount, or overall tenderness. However, sensory panelists rated steaks from the sequential treatment (RH + ZH) as having greater (*P < 0.05*) myofibrillar tenderness than steaks from all other treatments. To compliment this, steaks from the RH + ZH treatment had the lowest numerical (*P = 0.12*) WBSF values. In contrast to our findings, Holmer et al. (2009b) reported that *psoas major* steaks from cows supplemented with RH tended (*P = 0.10*) to have higher WBSF values. No other published literature has looked at the influence of β-AA in cull cow feeding systems on *psoas major* tenderness. Wheeler and Koohmaraie (1992) found that fractional degradation rates of myofibrils in β-AA fed steers began to decline after 1 wk, became significantly lower than controls at 3 wk, but were similar to controls by 6 wk of β-AA feeding. In my study, sequential feeding of RH followed by ZH consisted of a total of 45 d of β-AA treatment. It is possible that protein degradation rates in the *psoas major* had returned to normal after 45 d of β-AA supplementation, alleviating any negative contributions to tenderness. However, if this were the case, I would expect the sequential treatment to have similar tenderness values to controls rather than improved values, and I would expect similar results in other muscles.

Non-enhanced, longissimus sensory characteristics

Least squares means for base WBSF measurements of non-enhanced LM steaks are reported in Table 4.1. Cows in the ZH treatment tended *(P = 0.12)* to have higher WBSF values than cows supplemented with RH (5.5 vs. 4.1 kg). In a similar cull cow realimentation study, Hutchison (2007) reported that sensory panelists rated ZH supplemented cows as tougher than non-supplemented cows, and that ZH treatment yielded the highest WBSF values. Zilpaterol-HCl is generally considered a more potent β-AA than RH because it predominately binds $β_2$ receptors, the most abundant receptor subtype in beef skeletal muscle and adipose tissue (Sillence and Matthews, 1994). Therefore, it would be anticipated that RH would have less of an effect on tenderness than ZH. In agreement with our results, Holmer et al. (2009b) reported that feeding RH

to cows had no adverse affects on WBSF values of LM steaks. Additionally, it has been reported, that in steers and heifers, RH supplementation slightly increases WBSF values, but the impact on consumer acceptability is minimal (Platter et al., 2008). However, steaks from steers and heifers supplemented with ZH and subjected to 14 d of aging had increased WBSF values (0.4 to 1.3 kg) compared to controls (Claus et al., 2010; Hilton et al., 2010; Kellermeier et al., 2009).

To measure the extent of postmortem proteolysis, desmin degradation was measured. After 10 d and 21 d of aging, steaks from ZH and RH + ZH treatments had a lower ($P < 0.0001$) percentage of degraded desmin than steaks from C cows or cows supplemented with RH only. As expected, for all treatments, there was a higher percentage of degraded desmin after 21 d of postmortem aging than at 10 d. In contrast to my results, Kellermeier et al. (2009) reported that ZH supplementation in steers did not alter desmin degradation in LM steaks aged for 7, 14 and 21 d. However, in support of my results, Strydom and Smith (2010) reported increased calpastatin activity in steaks from β-AA supplemented steers. I speculate that desmin degradation was lower in ZH-fed cattle because of increased muscle calpastatin activity, and, thereby, reduced postmortem proteolysis.

Collagen assays were conducted on a subsample (9 per treatment) of LM steaks. There was no difference in total collagen as a result of β-AA supplementation. Kellermeier et al. (2009) reported that total collagen decreased in ZH supplemented cattle and in conjunction with this, muscle fiber diameter was also increased likely yielding a dilution effect in collagen concentration. In my study, there was no significant increase in LM area. Therefore, muscle hypertrophy in ZH supplemented cows was not sufficient enough to yield a difference in total collagen content. However, collagen solubility in ZH treated cows (ZH and RH + ZH treatments) was increased compared to control cows, and it was increased ($P < 0.05$) in the RH + ZH treatment compared to RH cows. Similar to effects observed in the *infraspinatus* muscle, I speculate that this is due to a higher percentage of newly synthesized collagen.

Enhanced longissimus sensory characteristics

Longissimus roasts were enhanced with a 0.1 M calcium lactate solution at 7 d postmortem. After enhancement, steaks were cut and aged for a total of 14 d before WBSF and sensory characteristics were evaluated. Least squares means of enhanced LM steaks are reported in Table 4.1. Sensory panel ratings and WBSF values indicate no differences in enhanced LM tenderness among feeding treatments. Therefore, even though there was a difference in postmortem proteolysis and non-enhanced LM tenderness, it appears that enhancement with calcium lactate enhancement diminished the ZH treatment effect.

Direct comparisons between non-enhanced and enhanced WBSF cannot be performed because of experimental design. However, when a change in WBSF value (delta WBSF) was calculated, there was a trend (*P = 0.08*) for greater reduction in WBSF values due to calcium lactate enhancement in the ZH treatment. It is possible that the calcium level in the enhancement solution overcame the calpastatin inhibition of postmortem proteolysis. Similar results were observed by Koohmaraie and Shackelford (1991) who found that calcium chloride infusion post-harvest can overcome the β-AA-induced toughness in lambs.

Sensory panelists rated beef flavor (*P < 0.05)* in RH and RH + ZH treatments as more intense than steaks from the ZH treatment. Pump percentages, although not significant, were numerically the lowest for RH and RH + ZH treatments and the highest for the ZH treatment, even though all treatments were pumped to a target 11%. This might have contributed to the differences in beef flavor intensity ratings. Off-flavors were most intense in cows not receiving β-AA supplementation, and RH and RH + ZH treatments had less (*P < 0.05)* intense off-flavors than control cows.

Ground beef color

Ground beef (85/15) packaged in PVC overwrap was subjected to 72 h of retail display. There was no main effect for treatment or hour of display by treatment interaction observed for ground beef initial color, subjective color scores, instrumental color values, or lipid oxidation measurements (Table 4.2). Initial color of ground beef was characterized as moderately light cherry red to cherry red for all treatments. There

was a trend (*P = 0.09*) for a main effect of treatment on instrumental L* values. Ground beef from the RH + ZH treatment tended (*P* = 0.09) to have higher L* values throughout retail display compared to ground beef from the RH treatment. As expected, there was an hour of display main effect indicating that throughout display, color deteriorated for ground beef in all treatments (Table 4.3). After 72 h of retail display, ground beef was rated as slightly dark red or reddish tan with approximately 20 to 39% surface metmyoglobin.

Hutchison (2007) and Gonzalez et al. (2009) reported that by d 5 of retail display, β-AA supplementation had detrimental effects on color shelf-life. However, other studies have reported that in both the *semimembranosus* and LM of young feedlot cattle supplemented with ZH for 20 or 30 d had similar, if not better, color shelf-life than controls (Gunderson et al., 2009; Hilton et al., 2009). I anticipate that transitions in muscle fiber type from oxidative to more glycolytic due to β-AA supplementation can influence beef color, but that other factors might have more of an impact on beef color stability than β-AA supplementation.

SUMMARY

Sensory characteristics of muscles from cull cows responded differently to β-AA supplementation. Feeding cows RH, ZH or a sequence of RH + ZH improved tenderness in the *infraspinatus.* In the *psoas major*, sequential feeding of β-AA improved sensory panel myofibrillar tenderness ratings. Supplementation of cull cows with ZH, either alone or in sequence with RH, tended to decrease tenderness in the LM due to decreased (*P* < 0.05) postmortem proteolysis. Yet, positive effects were observed on collagen solubility in the LM due to β-AA supplementation. Enhancement of LM steaks with calcium lactate can alleviate negative tenderness attributes associated with decreased postmortem proteolysis of β-AA supplemented cows. Sequential feeding of RH followed by ZH yielded no detrimental effects on color or sensory properties of meat from mature cows compared to feeding a single β-AA with a trend for greater response to calcium lactate enhancement.

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Table 4.1 Least squares means for sensory characteristics of *psoas major*, *infraspinatus*, non-enhanced *longissimus*, and calcium lactate enhanced *longissimus* steaks from mature cows fed a high-energy ration for 74 d with or without β-adrenergic agonist supplementation

Trait	Treatment[1]				SE	P-value
	C	RH	ZH	RH + ZH		
Infraspinatus WBSF, kg	4.4[b]	3.8[a]	4.0[a]	3.8[a]	0.17	0.04
Psoas major						
WBSF, kg	2.9	3.2	3.0	2.8	0.11	0.12
Myofibrillar tenderness[2]	7.2[a]	7.3[a]	7.2[a]	7.5[b]	0.08	0.03
Juiciness[3]	5.7	5.7	5.6	5.6	0.13	0.59
Beef flavor[4]	5.5	5.5	5.6	5.6	0.08	0.90
Connective Tissue[5]	7.6	7.6	7.6	7.6	0.08	0.77
Overall tenderness[2]	7.4	7.3	7.3	7.5	0.08	0.33
Off flavor[6]	6.9[a]	7.0[ab]	7.5[c]	7.1[b]	0.14	0.03
Non-enhanced *longissimus*						
WBSF, kg	4.5	4.1	5.5	4.8	0.38	0.12
Desmin[7] - 10 d, degraded %	40.8[b]	39.8[b]	17.3[a]	23.9[a]	3.72	<0.0001
Desmin[7] - 21 d, degraded %	58.1[b]	54.1[b]	32.9[a]	34.7[a]	4.34	<0.0001
Total Collagen, mg/g	6.11	6.09	5.32	5.61	0.471	0.55
Soluble Collagen, %	4.74[a]	6.12[ab]	6.90[bc]	7.91[c]	0.607	0.03
Enhanced *longissimus*[8]						
Pump, %	11.1	10.9	13.1	10.9	0.69	0.13
WBSF, kg	4.1	4.0	4.4	4.3	0.24	0.60
Myofibrillar tenderness[2]	5.4	5.2	4.8	5.0	0.21	0.19
Juiciness[3]	5.6	5.5	5.4	5.5	0.13	0.73
Beef flavor[4]	5.3[ab]	5.4[bc]	5.2[a]	5.5[c]	0.08	0.05
Connective Tissue[5]	5.9	6.0	5.9	6.5	0.32	0.46
Overall tenderness[2]	5.4	5.3	5.0	5.1	0.21	0.57
Off flavor[6]	6.8[a]	7.3[b]	7.0[ab]	7.2[b]	0.13	0.01
Delta WBSF[9]	0.4	0.1	1.1	0.5	0.29	0.08

[1]Treatments: C = concentrate fed for 74 d; RH = concentrate fed for 49 d then supplemented with ractopamine-HCl for 25 d; ZH = concentrate fed for 51 d then supplemented with zilpaterol-HCl for 20 d; RH + ZH = concentrate fed for 26 d then supplemented with ractopamine-HCl for 25 d followed by zilpaterol-HCl for 20 d
[2]Myofibrillar and overall tenderness: 8 = extremely tender, 1 = extremely tough
[3]Juiciness: 8 = extremely juicy, 1 = extremely dry
[4]Beef flavor: 8 = extremely intense, 1 = extremely bland
[5]Connective tissue: 8 = none, 1 = abundant
[6]Off-flavor: 8 = none, 1 = extremely intense

[7]Desmin: percentage degraded at 10 or 21 d of postmortem aging
[8]Enhanced with a 0.1 M calcium lactate solution at 7 d postmortem to a target 11% pump and then aged for a total of 14 d postmortem
[9]Change in WBSF values between non-enhanced and enhanced *longissimus* steaks

Table 4.2 Least squares means of β-adrenergic agonists effects on color characteristics of ground beef from mature cows fed a high-energy ration for 74 d with or without β-adrenergic agonist supplementation and subjected to 72 h of retail display

Trait	Treatment[1]				SE	P-value
	C	RH	ZH	RH + ZH		
Initial Color[2]	3.4	3.6	3.5	3.3	0.13	0.39
Display Color[3]	3.9	3.9	3.7	3.6	0.16	0.50
Discoloration[4]	1.8	1.6	1.9	1.8	0.17	0.53
L*	49.9	49.0	49.9	50.6	0.40	0.09
a*	21.6	22.2	22.1	21.9	0.56	0.85
b*	20.9	20.9	21.2	21.0	0.27	0.90
Saturation index[5]	30.2	30.6	30.7	30.5	0.57	0.93
Hue angle[6]	45.4	44.3	44.9	44.9	0.53	0.60
TBARS[7]	0.3	0.3	0.3	0.5	0.03	0.87
Initial	0.3	0.3	0.3	0.5	0.03	0.87
End of Display	1.3	1.2	1.5	1.5	0.14	0.04

[1]Treatments: C = concentrate fed for 74 d; RH = concentrate fed for 49 d then supplemented with ractopamine-HCl for 25 d; ZH = concentrate fed for 51 d then supplemented with zilpaterol-HCl for 20 d; RH + ZH = concentrate fed for 26 d then supplemented with ractopamine-HCl for 25 d followed by zilpaterol-HCl for 20 d
[2]Initial color: 1 = Bleached, pale red, 5 = Slightly dark red, 8 = Very dark red
[3]Display color: 1 = Very bright red, 5 = Slightly dark red or reddish tan, 7 = Tan to brown
[4]Discoloration: 1 = None (0%), 5 = Moderate (60-79%), 7 = Total (100%).
[5]Saturation index calculated using $(a^2 + b^2)^{1/2}$
[6]Hue angle calculated using $(b*/a*)^{tan-1}$
[7]Thiobarbituric acid reactive substances: mg malonaldehyde per 1 g meat

Table 4.3 Least squares means of display time effects on color characteristics of ground beef from mature cows fed a high-energy ration for 74 d with or without β-adrenergic agonist supplementation subjected to 72 h of retail display

Trait	Hour of Display							SE	P-Value
	0	12	24	36	48	60	72		
Display Color[1]	2.0^a	3.0^b	3.4^c	3.8^d	4.0^d	4.9^e	5.3^f	0.11	<0.0001
Discoloration[2]	1.0^a	1.1^a	1.3^a	1.9^b	2.0^b	2.3^c	2.8^d	2.29	<0.0001
L*	54.4^a	50.5^b	49.4^c	49.1^{cd}	48.6^d	49.5^d	47.7^e	0.33	<0.0001
a*	33.2^a	26.2^b	24.4^c	21.2^d	20.2^e	18.0^f	10.4^g	0.34	<0.0001
b*	27.7^a	22.7^b	21.7^c	20.1^d	19.8^d	18.6^e	16.3^f	0.19	<0.0001
Saturation index[3]	43.2^a	34.6^b	32.6^c	29.2^d	28.3^e	25.9^f	19.4^g	0.37	<0.0001
Hue Angle[4]	39.8^a	40.9^b	41.7^c	43.5^d	44.5^e	46.1^f	57.6^g	0.36	<0.0001

[1]Visual color: 1 = Very bright red, 5 = Slightly dark red or reddish tan, 7 = Tan to brown
[2]Discoloration: 1 = None (0%), 5 = Moderate (60-79%), 7 = Total (100%).
[3]calculated using $(a^2 + b^2)^{1/2}$
[4]calcualted using $(b*/a*)^{tan-1}$

Chapter 5 - General Conclusions and Implications

The use of β-adrenergic agonists (β-AA) in young cattle has profound effects on feedlot performance and carcass characteristics. My results, and previously published studies, indicate that β-AA supplementation has less pronounced effects in mature cows. The lack of responsiveness to β-AA supplementation in mature cows could be an effect of decreased $β_2$-adrenergic receptor density ($β_2$-AR) with increased animal age. Some researchers have found trends for RH supplementation to increase the expression of $β_2$-AR mRNA in steers, heifers, and cull cows. Therefore, one of the main objectives of our study was to determine if feeding ractopamine-HCl (RH) prior to zilpaterol-HCl (ZH) supplementation would up-regulate $β_2$-AR density and increase responsiveness to ZH supplementation. I found that RH did not increase $β_2$-AR mRNA expression in the sequential treatment, but that both RH and ZH supplementation in the last 20 to 25 d of feeding increased $β_2$-AR mRNA. I am uncertain as to why RH supplementation did not increase $β_2$-AR mRNA earlier in feeding, but did during the later stage of feeding. Nonetheless, the aim of up-regulating $β_2$-AR density in the sequential treatment was not accomplished.

However, several aspects of our data offer valuable information regarding cellular and muscle responses to β-AA supplementation in mature cows. Feeding ZH, alone or following RH supplementation, increased Type IIx myosin heavy chain (MHC-IIx) mRNA expression. This indicates that ZH supplementation might cause a transition in muscle fiber types from slow-twitch (Type I) towards fast-twitch muscle fibers (Type-IIx). This transition could not only influence growth potential, but could also affect sensory properties of meat. Type II muscle fibers are larger in diameter and utilize glycolytic rather than oxidative metabolism. The increased diameter of Type II muscle fibers might have contributed to the numerical increase in LM area in the ZH and RH + ZH treatments. Increased fiber diameter of Type II muscle fibers might also have contributed to decreased tenderness in the LM of ZH supplemented cows.

Probably a larger contributor to increased body weight gain, HCT, LM area and decreased tenderness in ZH supplemented cows was decreased protein degradation which carried over into decreased postmortem proteolysis. Desmin degradation

analysis in my study indicated that ZH supplementation decreased the extent of postmortem proteolysis in the LM muscle at both 10 and 21 d postmortem compared to control and RH treatments. Protein degradation appears to be reduced more with ZH supplementation than with RH supplementation resulting in greater improvements in live animal and carcass characteristics, but also carrying over into negative attributes in meat.

Postmortem proteolysis is largely regulated by calcium activated enzymes that degrade cytoskeletal proteins. Our aim to improve tenderness of β-AA supplemented cows by enhancement with calcium lactate was successful. Therefore, we can postulate that enhancement with calcium lactate promotes the activation of enzymes counteracting the decreased proteolysis caused by ZH supplementation.

Few studies have looked at collagen characteristics in β-AA supplemented cattle. Our data showed that the percentage of soluble collagen in the LM is increased by RH or ZH supplementation, with ZH having a more pronounced effect. This suggests that protein synthesis is not only increased in skeletal muscle, but also in connective tissue of β-AA supplemented cattle. Although increased collagen solubility yielded no improvement in LM tenderness of ZH supplemented cows, we postulate that collagen solubility did offer a benefit in *infraspinatus* tenderness because the *infraspinatus* contains a higher percentage of collagen compared to the LM.

From an economic standpoint, it was beneficial to implant and feed cows a high-energy diet. Even though not significant, ZH supplementation yielded higher net revenue because of numerically greater live weights at the end of feeding. The use of β-AA in cull cow realimentation programs does not offer additional benefits compared to just implanting when feeding a high-energy diet, partially due to the large amount of variation that exists in cull cows from different backgrounds. However, cull cows also appear to respond differently to β-AA supplementation than young cattle. Future research should explore mechanistic reasons as to why β-AA are not as effective in mature cattle populations.

Appendix A - Muscle Biopsy Procedure

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Adapted from:

Dunn, J. D., B. J. Johnson, J. P. Kayser, A. T. Waylan, E. K. Sissom, and J. S. Drouillard. 2003. Effects of flax supplementation and combined trenbolone acetate and estradiol implant on circulating insulin-like growth factor-I and muscle insulin-like growth factor-I messenger RNA levels in beef cattle. J. Anim. Sci. 81:3028-3034.

Pampusch, M. S., M. E. White, M. R. Hathaway, T. J. Baxa, K. Y. Chung, S. L. Parr, B. J. Johnson, W. J. Weber, and W. R. Dayton. 2008. Effects of implants of trenbolone acete, estradiol, or both, on muscle IGF-I, IGF-I receptor, estrogen receptor-α and androgen receptor mRNA levels in feedlot steers. J. Anim. Sci. 86:3418-3428.

Winterholler, S. J., G. L. Parsons, D. K. Walker, M. J. Quinn, J. S. Drouillard, and B. J. Johnson. 2008. Effect of feedlot management system on response to ractopamine-HCl in yearling steers. J. Anim. Sci. 86:2401-2414

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Supplies Needed

Squeeze chute	Gauze (sterile)
Clippers with surgical blades	Gloves (sterile)
Gloves (non-sterile)	Novasaine
Betadine surgery scrub	Sterile surgery tray
Scrub brushes	Scalpel holder with a number 22 blade
Ethanol (70%)	Bergstrom biopsy needle and plunger
Gauze (non-sterile)	Forceps
Disposable razors	Aerosol Spray bandage
Lid to mark site	Tissue glue
Paint marker to mark site	Whirlpack bags
Disposable syringe (12 cc)	Liquid nitrogen
Needles (20 gauge)	Cooler with Dry ice
Lidocaine	

Protocol

1. Restrain cow or calf in a hydraulic squeeze chute.

2. Clip hair between the 10th and 13th rib with clippers equipped with surgical blades.

3. Rinse the clipped area with Betadine and scrubbed in a circular motion for 5 min.

4. Use a disposable razor to remove any remaining hair.

5. Use ethanol to wash away Betadine and loose hair.

6. Scrub area with Betadine for an additional 2-3 min and rinse with ethanol.

7. Use a lid or other circular shape and mark biopsy site with paint marker.

8. Subcutaneously inject 2 cc of Lidocaine into four spots (total of 8 cc) surrounding the marked biopsy site. After approximately 5-8 min, non-response to needle prick will be used to determine if the spot is properly numbed.

9. Wipe the biopsy site an additional time with ethanol and sterile gauze.

10. While wearing sterile gloves, make a 1-cm incision.

11. Use a Bergstrom biopsy needle (6-mm) to collect ~1.5 g of muscle tissue.

12. Place muscle tissue in a whirlpack bag, snap freeze in liquid nitrogen, and place in a cooler containing dry ice.

13. Wipe away excess blood from the incision with sterile gauze.

14. Close the incision using 2 to 3 drops of tissue adhesive.

15. Cover the incision site with an aerosol spray bandage.

16. Monitor incision cite for swelling and post biopsy complications.

Appendix B - RNA Isolation Procedure

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Adapted from:

Winterholler, S. J., G. L. Parsons, D. K. Walker, M. J. Quinn, J. S. Drouillard, and B. J. Johnson. 2008. Effect of feedlot management system on response to ractopamine-HCl in yearling steers. J. Anim. Sci. 86:2401-2414

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Supplies Needed

Scale to weigh tissue	Cold 75% ethanol
Plastic weight boats to weigh tissue	DNase-RNase-free water
Autoclaved forceps	Polypropylene test tubes w/o cap
Chemical hood	1.5-ml microcentrifuge tubes
Homogenizer	0.5-ml microcentrifuge tubes
Ice	DNase-RNase-free pipet tips 10 µL
TRIzol Reagent	DNase-RNase-free pipet tips 200 µL
Chloroform	DNase-RNase-free pipet tips 1000 µL
Micropipetters	RNaseZap
Isopropyl alcohol	

Protocol

*Steps involving TRIzol reagent or chloroform should be performed in a chemical hood.

Day 1:

1. Weigh frozen tissue (~100 mg).

2. Place tissue in polypropylene tube containing 1 ml of ice cold TRI Reagent.

3. Homogenize 100 mg of tissue in 1.0 mL TRIzol Reagent for at least 30 s. Make sure you cannot visibly see tissue after homogenizing.

4. Transfer homogenate to autoclaved 1.5 mL microcentrifuge tube.

5. Incubate homogenate for 5 min at ambient temperature to allow dissociation of nucleoprotein complexes.

6. Add 0.2 ml chloroform per 1 mL TRIzol used in homogenization (add 0.2 mL chloroform to 1 mL TRIzol in each of your tubes). Chloroform is at room temperature.

7. Cap sample vortex for 15-30 s.

8. Incubate samples for 3 min at ambient temperature.

9. Centrifuge at no more than 12,000 x g for 15 min at 4°C.

10. Transfer the aqueous phase (top, clear) to an autoclaved 1.5 mL microcentrifuge tube. Remove the aqueous phase until you barely reach the interphase (be careful that you do not disrupt the layers). Put samples on ice until you are finished with your 2 or 4 samples.
    a. Upper colorless aqueous phase- contains total RNA
    b. Interphase (white disc)- contains DNA
    c. Lower red organic phase- contains DNA and protein
Dispose of organic phase in appropriate waste container in chemical hood.

11. Mix aqueous phase with isopropyl alcohol (2-propanol) in a 1:1 ratio to precipitate RNA (add 550-600 µL of isopropyl alcohol). Isopropyl alcohol is at RT.   Mix well or vortex.

12. Incubate samples for 10 min at ambient temperature.

Optional to increase RNA yield- incubate samples at -80°C for ten min and incubate an additional h to overnight (8-12 h incubation) at -20°C.

Day 2:

13. Thaw samples on ice for 15 min.

14. Centrifuge at no more than 12,000 x g for 10 min at 4°C.

15. Remove supernatant.

16. Decant off majority of supernatant (into spare tube).

17. Pour off supernatant with pellet at top of tube.

18. Remove excess supernatant with pipette.

19. Can re-centrifuge to re-pellet RNA pellet if needed.

20. Wash RNA pellet with 1 ml 75% ice cold ethanol per 1 ml TRIzol used in homogenization (1 mL 75% ethanol). Wash pellet by pipetting ethanol over pellet at least 6-8 times.

21. Mix sample by vortexing lightly.

22. Centrifuge at 7,500 x g for 5 min at 4°C (8500 rpm; *note- can go up to 12,000 x g*).

23. Remove supernatant as before.

24. Air-dry until wet appearance is gone (5 to 10 min); do NOT vacuum-dry. If too dry, re-suspension will be difficult

25. Dissolve RNA in DNase-RNase-free water (approximately 50-100 μl per 100 mg tissue) by passing solution through pipette tip.

26. Amount of water to use depends on:
    a. amount of RNA needed for a reaction
    b. concentration needed for a reaction
    c. solubility of the RNA pellet.

27. Lightly vortex the tube.

28. Transfer the contents into a sterile 1.5 mL centrifuge tube. Make sure this tube is labeled very well.

29. Freeze all samples at -80°C.

Appendix C - DNAse Procedure

Supplies Needed
TURBO DNA-free Kit
Microcentrifuge tubes (0.5 mL)
Nuclease-free water

Protocol
1. Prepare Master Mix (on ice):

	1 Tube
10x Turbo DNase Buffer	1.2 ul
TURBO DNase	0.5 ul
ddH$_2$O	2.8 ul
Total	4.5 ul

2. Add 4.5 µL master mix to each tube.

3. Add 7.5 µL RNA to each tube.

4. Incubate 40 min at 37˚C.

5. Vortex the DNAse inactivation reagent.

6. Add 4 µL of DNAse inactivation reagent to each tube and finger click to mix.

7. Incubate 3 min at room temperature.

8. Finger flick several times to disperse the inactivation reagent.

9. Centrifuge for 1.5 min at 10,000 rpm.

10. Carefully transfer the supernatant containing the RNA to a new tube. Avoid the inactivation reagent, as it can interfere with downstream reactions.

11. Final samples volume should be 10-12 uL.

12. Store in the -80 freezer. DNase treated RNA is unstable.

Appendix D - Agilent RNA 6000 NanoAssay Protocol

Preparing the Gel
1. Pipette 500 µL of RNA 6000 Nano gel matrix into a spin filter.
2. Centrifuge at 1500 g $\pm$ 20% for 10 min at room temperature.
3. Aliquot 65 µL filtered gel into 0.5 mL microcentrifuge tube. Use within 4 weeks.

Preparing the Gel-Dye Mix
1. Allow dye concentrate to equilibrate to room temperature for 30 min.
2. Vortex dye concentrate for 10 sec., spin down, and add 1 µL of dye into a 65 µL aliquot of filtered gel.
3. Vortex solution well. Spin tube at 13000 g for 10 min at room temperature. Use within one day.

Loading the Gel-Dye Mix
1. Put a new RNA 6000 Nano chip on the chip priming station.
2. Pipette 9.0 µL of gel-dye mix in the well marked (G).
3. Make sure that the plunger is positioned at 1 mL and then close the chip priming station.
4. Press the plunger until it is held by the clip.
5. Wait for exactly 30 s and then release the clip.
6. Wait for 5 s. Slowly pull back the plunger to the 1 mL position.
7. Open chip priming station and pipette 9.0 µL of gel-dye mix in the wells marked (G).
8. Discard the remaining gel-dye mix.

Loading the Agilent RNA 6000 Nano Marker
1. Pipette 5 µL of RNA 6000 Nano marker in all 12 sample wells and in the well marked (ladder).

Loading the Ladder and Samples
1. Pipette 1 µL of prepared ladder in well marked (ladder).
2. Pipette 1 µL of sample in each of the 12 sample wells. Pipette 1 µL of RNA 6000 Nano Marker in each unused sample well.
3. Put the chip in the adapter of the IKA vortexer and vortex for 1 min at 2400 rpm.
4. Run the chip in the Agilent 2100 bioanalyzer within 5 min.

Appendix E - Reverse Transcription Procedure

Reagents needed per sample

5 uLs	10x Buffer	RT Mix = 32.6 uL
11 uLs	$MgCl_2$	RNA & Water = 17.4 uL
10 uLs	dNTP	Total = 50uLs / PCR Tube
2.5 uLs	Hexamer	
1 uL	Inhibitor	
3.125 uLs	Multi-scribe	
32.625 uLs	Total	

Protocol

1. Mix reagents in microcentrifuge tube.

2. Label reaction tubes.

3. Add appropriate amounts of sample using the post DNased conc.

4. Add nuclease free water (17.4 – x = nuclease free water).

5. Add 32.6 uLs of reagent mixture (never go phase last click on pipette).

6. Close and flick reaction tubes gently!!!

7. Centrifuge 5 sec.

8. Put in RT machine. RT-PCR cycle: $25^{o}C$;10min, $37^{o}C$;60min, $95.5^{o}C$;5min, $4^{o}C$;60min.

9. When samples are done, place in – 80 freezer.

Appendix F - Real-time PCR Procedure

1. Place samples, primers, and probes on ice to thaw.

2. Calculate amounts needed for each primer and probe.

3. Mix the following in separate tubes:

   Ex: # of samples + 1 NTC x 3 wells
       12 samples + 1 = 13 x 3 = 39 wells (use 44 to be careful)
   <u>MHC-I</u>
   10 uM Forward Primer   2.25 uLs x 44 = 99 uLs
   10 uM Reverse Primer   2.25 uLs x 44 = 99 uLs
   10x Probe   0.5 uLs x 44 = 22 uLs (2.2 uL probe + 19.8 uL Nuc Free H_2O)
   Nucrease Free Water   6.5 uLs x 44 = 286 uLs
                     Add 11.5 uLs/sample of above

   <u>18s</u>
   Primer & Probe   1.25 uLs x 44 = 55 uLs
   Nuclease Free Water   10.25 uLs x 44 = 451 uLs
                      Add 11.5 uLs/sample of above

4. Remove Master Mix from refrigerator.

5. Label tubes for each sample including NTC.

6. Mix 9 uLs of sample + 112.5 Master Mix = 121.5/9 = 13.5 uLs.
   **13.5 uLs is the constant that you always want to place in each well.

7. For NTC use 9 uLs Nuclease Free Water + 112.5 Master Mix.

8. Flick all tubes to ensure they are mixed then place back on ice.

9. Remove plate and begin plating with NTC's on top row.

10. Plate Primers and Probes first – 11.5 uLs/ well.

11. Added 13.5 uLs of sample to each of 6 wells (run each in triplicate)

12. Make sure all wells are filled and there are no air bubbles.

13. Place plastic tape over top and seal with rubber card.

14. Put in PCR machine and place rubber matt on top, close machine and begin running program.

Appendix G - Carcass Data Descriptors

Color of Lean

7 = Very light cherry red
6 = Cherry red
5 = Slightly dark red
4 = Moderately dark red
3 = Dark red
2 = Very dark red
1 = Black

Fat Color

5 = Canary yellow
4 = Yellow
3 = Slightly yellow
2 = White
1 = Bleached white

Maturity

100 = A-00
200 = B-00
300 = C-00
400 = D-00
500 = E-00

Appendix H - Sensory Panel Descriptors

Myofibrillar Tenderness
1 = Extremely tough
2 = Very tough
3 = Moderately tough
4 = Slightly tough
5 = Slightly tender
6 = Moderately tender
7 = Very tender
8 = Extremely tender

Beef Flavor Intensity
1 = Extremely bland
2 = Very bland
3 = Moderately bland
4 = Slightly bland
5 = Slightly intense
6 = Moderately intense
7 = Very intense
8 = Extremely intense

Overall Tenderness
1 = Extremely tough
2 = Very tough
3 = Moderately tough
4 = Slightly tough
5 = Slightly tender
6 = Moderately tender
7 = Very tender
8 = Extremely tender

Juiciness
1 = Extremely dry
2 = Very dry
3 = Moderately dry
4 = Slightly dry
5 = Slightly juicy
6 = Moderately juicy
7 = Very juicy
8 = Extremely juicy

Connective Tissue Amount
1 = Abundant
2 = Moderately abundant
3 = Slightly abundant
4 = Moderate
5 = Slight
6 = Traces
7 = Practically none
8 = None

Off-Flavor Intensity
1 = Abundant
2 = Moderately abundant
3 = Slightly abundant
4 = Moderate
5 = Slight
6 = Traces
7 = Practically none
8 = None

Appendix I - Protocol for Determining Desmin Degradation

METHOD USED TO EXTRACT TOTAL MUSCLE PROTEIN FOR WESTERN BLOT USING TRIS-EDTA BUFFER

SOLUTIONS FOR SAMPLE EXTRACTION

1. Tris-EDTA Buffer, pH 8.3

	1 L
50 mM Tris	6.06 g
10 mM EDTA	3.72 g

Adjust pH to 8.3; qs t o1 liter. Store at 4°C.

2. 0.5 M Tris, pH 6.8

	200 mL
Tris	12 g

Adjust pH to 6.8 with HCl; qs to 200 mL. Filter and store at 4°C.

3. 10% SDS

	500 mL
SDS	50 g

qs to 500 mL. Filter with Whatman filter paper. Store at room temperature.

4. Filtered Distilled Water

5. 8 mg/ml Bromophenol Blue (0.8%)

6. 2X Treatment Buffer minus MCE, pH 6.8

0.125 M Tris	2.5 mlL solution (2)	50 mL solution (2)
4% SDS	4.0 mL solution (3)	80 mL solution (3)
20% glycerol	2.0 mL	40 mL
10% MCE	---	---
H_2O	0.5 mL	10 mL
	9.0 mL	180 mL

Initial pH 7.1. pH to 6.8. Store at room temperature.

7.   2X Treatment Buffer + MCE 8 Bromophenol Blue, pH 6.8

          9.0 mL 2X Treatment Buffer (Solution 6)
          50 μL MCE
          500 μL Bromeophenol Blue (Solution 5)

          Make fresh daily (or use aliquots that have been frozen). Any solution not used may be aliquoted and frozen for further use.

SOLUTIONS FOR GEL ELECTROPHORESIS

8.   Stock Acrylamide (30% (37.5:1)

	100 mL
Acrylamide	29.2 g
Bisacrylamide	0.779 g

          Mix (wrap beaker in foil to prevent solution from light) and adjust to 100 mL. Filter and store in a dark bottle at 4°C.

Caution: Acrylamide is a neurotoxin. Wear gloves and face mask when working with it.Wash hands thoroughly after use. Polymerized gels can be disposed in the trash. Unpolymerized solutions are disposed as hazardous waste, contact disposal.

9.   1.5 M Tris base, pH 8.8

          18.15 g/100 mL ddH$_2$O   90.75 g/500 mL ddH$_2$O

          pH to 8.8 with HCl. Filter and store at 4°C.

10. 0.5 M Tris base, pH 6.8

          6 g/100 mL ddH$_2$O   30 g/500 mL ddH$_2$O

          pH to 6.8 with HCl. Filter and store at 4°C.

11. 10% SDS

          10 g/100 mL ddH$_2$O

          Filter (with filter paper) and store at room temperature up to 6 months. Some heat maybe required to dissolve. Wear a face mask when preparing this solution.

12. 10% Ammonium Persulfate

1g/10 mL ddH$_2$O

M. Koohmaraie's lab prepares this and stores it in a dark bottle at 4°C.

13. Running Buffer, pH 8.3

	10X
0.25 M Tris (F.W. 121.1)	30.0 g
1.92 M glycine	144.0 g
ddH$_2$O to 1 L	

It is not necessary to check the pH of this solution. Store at room temperature.

To make 1X Running Buffer:
100 mL of 10X solution
10 mL of 10% SDS
890 mL ddH$_2$O

Make fresh for each gel run. Do not reuse the running buffer. Reusing the buffer can affect reproducibility since the ionic strength and pH of the buffer will change during the run (per Bio-Rad).

14. Water Saturated Butanol

50 mL n-Butanol + 10 mL ddH$_2$O

Dispose excess as hazardous waste. Butanol that is used as gel overlay (approx.1 mL) may be poured onto a paper towel and then allowed to evaporate in the hood.

SOLUTIONS FOR PROTEIN TRANSFER

15. Transfer Buffer

	2L	4L
Glycine	28.83 g	57.66 g
Tris	6.06 g	12.12 g
10% Methanol	200 mL	400 mL
ddH$_2$O	to 2 L	to 4 L

Should be pH 8.1 – 8.3 without pHing. This solution may be reused 1-2 times. Dispose in sewer system.

16. 0.1% Amido Black Staining Solution

	100 mL
Amido Black	100 mg
10% Methanol	10 mL
2% Acetic Acid	2 mL
ddH$_2$O	88 mL

Mix and filter. Store at room temperature. Dispose in sewer system.

17. Destain

20% Methanol 2000 mL
7 % Acetic Acid 700 mL
ddH$_2$O 7300 mL
Dispose in methanol hazardous waste container.

SOLUTIONS FOR WERSTERN BLOT

18. TBS, pH 7.4

	1 L	or	10X	1 L
20 mM Tris	2.4 g			24 g
137 mM NaCl	8.0 g			80 g
5 mM KCl	0.2 g			2 g
(dilute to 1X to use)				

pH with 1 N HCl. Filter and store at room temperature.

19. TTBS (0.05% Tween 20), pH 7.4

Add 250 µL Tween 20 to 500 mL TBS.

20. Blocking Solution

2.5% Sheep Serum 12.5 mL of crude prep sheep serum
TTBS 5   00 mL

SAMPLE PREPARATION

1. Homogenize 1 g of sample in 10 volumes (10 mL) 1X extraction buffer (Solution 1) for 20 sec with the Polytron at setting #4. Do this step in the cold room if using raw samples. For cooked core samples, this step may be done in the lab.

2. Immediately remove a 0.5 mL aliquot for solubilization and transfer to a 1.5 mL microcentrifuge tube.

3. Add 0.5 mL of 2X Treatment buffer (minus MCE). Mix well by repeatedly pipetting. Nucleic acids may be stringy and viscous, but pipetting will help shear them. Vortex sample.

4. Heat samples in a 50°C water bath for 20 min., repeat mixing, and reheat for 5 min.

5. Centrifuge for 20 min in a Eppendorf 5414 C centrifuge (maximum setting = 16,000 x g), to pellet insoluble material. Pellet should be small or undetectable.

6. Determine protein concentration of the supernatant (diluted 1:5 with 1X Treatment Buffer (10 µL sample + 40 µL buffer) using the micro-BCA protein assay (use microtiter well plates). Do in triplicate.

   To each well add:
   > Sample: 10 µL diluted sample
   > > or
   > Standard: 10 µL (4, 2, 1, 0.5, 0 mg/mL BSA)

7. Add 200 µL BCA reagent and incubate at 37°C for 30 min. Read plate on the microplate reader at 562 nm. If more than one plate is used, run a standard curve with each plate. Mix standards to contain the same concentration of potential interfering substances as the samples.

8. Dilute samples to 3 mg/mL (or your desired protein concentration) using 2X treatment buffer containing MCE and bromophenol blue (Solution 7). Mix samples well and heat in a 50°C waterbath for 10 min prior to loading on gel. Samples may be frozen at this point if gels cannot be run at this time.

GEL ELECTROPHORESIS

1. The height of the separating gel is 5.5 cm. A 4% stacker is used.

		SEPARATING				5 % Continuous
	4 % Stacker	**15 %**	**12.5 %**	**10 %**	**7.5 %**	
1.5 M Tris, pH 8.8	---	5.0 mL	5.0 mL	5.0 mL	5.0 mL	5.0 mL
0.5 M Tris, pH 6.8	1.88 mL	---	---	---	---	---
30% Acrylamide	1.0 mL	9.98 mL	8.35 mL	6.65 mL	4.99 mL	3.3 mL
10% SDS	0.075 mL	0.2 mL	0.2 mL	0.2 mL	0.2 mL	0.2 mL
Filtered ddH$_2$O	4.55 mL	4.72 mL	6.35 mL	8.05 mL	9.71 mL	11.2 mL
10% APS	50 µL	100 µL	100 µL	100 µL	100 µL	100 µL
TEMED	7.5 µL	10 µL	10 µL	10 µL	10 µL	10 µL

2. Mix separating gel and degas 15 min. Add TEMED.

3. Pour gel (5.5 cm); overlay with water saturated Butanol and allow to polymerize 1 hr.

4. Make stacking gel and degas 15 min. Add APS and TEMED and mix immediately before stacker is to be poured (see step 5).

5. Pour off water saturated Butanol and rinse well with distilled water. Remove any residual water with a Kimwipe. Pour stacker making sure that no air bubbles are trapped under the wells. Place comb in between plates. Allow to polymerize 30-45 min.

6. Carefully remove comb and rinse wells with water. Remove residual water with a Kimwipe. Assemble gel rig using running buffer in the lower chamber and running buffer in the upper chamber.

7. If samples were frozen, heat thawed samples in a 50°C water bath for 5 min.

8. Run gels at 200 volts for 45 min or until dye front just runs off the end of the gel.

9. While gel is running, prepare everything to transfer proteins from the gel to the membranes.

SAMPLE LOADING

1. A standard is run on every gel in triplicates. The standard preferably is a pooled sample of multiple animals collected at 0 h postmortem. Based on the samples to be run, the standard must be species and muscle specific.

2. Always leave one or two outside lanes open (to avoid sample smiling). The standard is run in the outside lanes and the middle lane (see example).

Lane	
1	PDB
2	**0 h standard**
3	Sample
4	Sample
5	Sample
6	Sample
7	Sample
8	**0 h standard**
9	Sample
10	Sample
11	Sample
12	Sample
13	Sample
14	**0 h standard**
15	PDB

PROTEIN TRANSFER

1. Cut blotter paper (Whatman 3MMChr Chromatography paper) to 3 in x 4 in. Cut the PVD Membranes to 2.5 in x 3.5 in. Notch the upper left corner of the membrane. This notch will correspond to lane 1 of the gel. Using a pencil, mark your ID in this corner. Be careful to never touch the membranes with your hands – always wear gloves. Handle membranes with forceps.

2. Place transfer buffer in a tray. Assembling of the sandwiches will take place in this tray. Lay 1.2 of the plastic cassette in the tray. Place 1 buffer saturated sponge on top of this.

3. Remove one gel from rig and remove one glass plate. Remove all stacker. You may have to rub the glass plate with your finger to insure that all the stacker has been removed. Notch the gel at lane 1. Slide the gel into the tray containing transfer buffer.

4. Wet blotter paper (2 sets, 2 papers each) in transfer buffer.

5. Fill one tray (pipet tip box lids) with methanol and another with distilled water. Wet one membrane in methanol for approximately 5 sec, making certain that the membrane is totally submerged. Transfer this membrane to the tray containing water for 30 s. Transfer to Transfer Buffer.

6. Transfer Stack Assembly. The sandwich is assembled so that the negative charge travels through the gel to the membrane. Assemble as follows: On top of the saturated sponge place 2 buffer saturated blotter papers. Remove air bubbles by rolling a 15 mL conical over the surface. Place the membrane on top of the blotter paper, curved side up. Make sure there are no air bubbles under the membrane. Center the gel on tip of the membrane. Gently remove air bubbles with your finger. Make certain your gloves are wet or the gel will tear. Place 2 buffer saturated blotter papers on the gel, from the center towards the edge. Roll a 15 mL conical over the paper surface to remove air bubbles. During this process, keep all surfaces wet. Place 1 buffer saturated sponge on top of the blotter paper. Place a plastic cassette on top of this. Place sandwich in tank with the gel on the negative electrode side (black = negative; red = positive). Fill tank with cold Transfer Buffer. Transfer is done at 4°C. Place the tank on a stir plate with gentle stirring.

7. Transfer. 10% gels transferred at 200 mA for 1 h at 4°C

8. Rinse blot in water for about 10 s and place in blocking solution and store overnight at 4°C.

WESTERN BLOTTING PROTOCOL FOR PIERCE SUPERSIGNAL WEST DURA EXTENDED DURATION SUBSTRATE

10 mL sufficiently covers 1 membrane.

1. Perform electrophoresis and transfer as listed in the protocol, Method Used to Extract Total Muscle Protein for Western Blot Using Tris-EDTA Buffer.

2. If blot was air dried, re-wet blot in Methanol and then water.

3. Non-specific binding sites are blocked by immersing the membrane in TTBS + 2.5 Sheep Serum for 1 h at room temperature on an orbital shaker. Membranes may be left in the blocking solution overnight in a refrigerator.

4. During the blocking step, dilute the primary antibody in TTBS. (1:300 D_3 specific for desmin)

5. Incubate the membrane in diluted primary antibody for 1 h at room temperature.

6. Filtered TTBS (0.05% Tween) is used for the washes. Using a squirt bottle filled with TTBS, briefly rinse the membrane using the 2 changes of TTBS. Wash once for 15 min and twice for 5 min with fresh changes of TTBS at room temperature.

7. During the washing step, dilute the secondary antibody in TTBS (1:10,000 anti-mouse). Use the Pierce ECL antibody anti-mouse.

8. Incubate the membrane in diluted secondary antibody for 1 h at room temperature.

9. Using 20 mL/membrane wash the membrane 2 x 15 min and 4 x 5 min in fresh changes of TTBS.

10. Detection.

DETECTION

It is necessary to work quickly once the membranes have been exposed to the detection solution.

1. Using the Pierce SuperSignal West Dura Extended Duration Substrate Kit, mix an equal volume of West Dura Lumino/Enhancer solution with West Dura Stable Peroxide Solution to give sufficient coverage of the membrane. 3.0 mL will cover one membrane. This substrate can be reused twice without significant loss of signal.

2. Place membrane in a tray, protein side up.

3. Add the detection solution and incubate for 5 min at room temperature without shaking.

4. Drain off excess detection reagent and transfer membrane to a sheet protector. Gently smooth out air pockets.

5. Place the membrane, protein side up, in the light box. Adjust the camera for enlargement, focus, and light.

6. Expose the membranes for the desired time (5 min). Check image exposure saturation after first exposure. If saturated, reduce exposure time and re-expose blot. Desired exposure time is reflective of minimal or no saturation across all bands on the blot.

7. Save the images after exposure is complete.

DATA ANALYSIS USING THE ALPHA INNOTECH IMAGE ANALYSIS SYSTEM

1. QUANTIFICAITON: Using the Spot Density function, draw tight boxes around the bands of interest. Do not use the auto background function. Do not use the enhance function unless it is a picture being used for publication purposes.

2. CALCULATIONS: Obtain the IDV mean for all three 0 h standard samples. Calculate the % desmin remaining using the formula: (protein IDV / mean of pooled 0 h standard IDV) x 100

OPTIMAL CONDITIONS FOR ECL ANTIBODY DETECTION PIERCE SUPER SIGNAL WEST DURA EXTENDED DURATION SUBSTRATE FOR DESMIN – D3

10% gel (30% acrylamide, 37.5:1)
Transfer 1 h at 4°C at 200 mA
Blocking agent: TTBS + 2.5% Sheep Serum
Protein concentration: 15 µg (15 well gel)
Primary dilution: 1:10 (will vary for each lot of D3)
Secondary dilution: 1:10,000 Pierce antibody, anti-mouse
ECL detection:
    Incubation: Pierce West Dura Substrate; 5 min incubation
    Exposure: 5 min exposure

Appendix J - Collagen Procedures

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Adapted from:

Cross, H. R., Z. L. Carpenter and G. C. Smith. 1973. Effects of intramuscular collagen and elastin on bovine muscle tenderness. J. Food Sci. 38:1961.

Hill, F. 1966. The solubility of intramuscular collagen in meat animals of various ages. J. Food Sci. 31:161.

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Solutions

1. <u>Ringers Solution (store up to 1 month in refrigerator)</u>

Sodium chloride   3.5 g
Calcium chloride   0.013 g
Potassium chloride 0.18 g

a. Dissolve and dilute to 500 mL with distilled water.
b. Dilute to ¼ Ringer's by mixing 1 part Ringer's with 3 parts ddH$_2$O.

2. <u>Oxidant solution</u>

<u>Buffer (store up to one month in refrigerator)</u>
    Sodium acetate   60.0 g
    Citric acid   13.0 g
    Sodium hydroxide   7.5 g

a. Dissolve in 200 mL of distilled water.
b. Add 145 mL isopropyl alcohol.
c. Dilute to 500 mL distilled water.

<u>Oxidant solution (make fresh daily)</u>
    Chloramine-T   1.41 g
    Buffer solution   100 mL

3. <u>Erhlich's Ragent</u>

<u>Dimethylaminobenzaldehyde solution</u>

    Dimethylaminobenzaldehyde   29.5 g
    Perchloric acid (70%)   27.7 mL
    Combine in beaker place in ice bath under exhaust hood.

Erhlich's

       Dimethylaminobenzaldehyde solution   44.2 mL
       Isopropyl alcohol   235.8 mL

4. Stock Standard Solution

       L-hydroxyproline   0.0250 g
       0.1 N HCl   5.0 mL

   a. Dissolve Hydroxyproline in 300 mL of water
   b. Add HCl
   c. Dilute to 500 mL with distilled water
   d. Dilute to 0, 5, 10, 15, 20, and 30 µg/mL

Protocol

Separation and hydrolysis of soluble and insoluble collagen

1. Weight 3 g of pulverized tissue in duplicate into centrifuge tubes.

2. Add 12 mL of heated ¼ strength Ringer's solution.

3. Place in 70°C waterbath fo r80 min. stirring every 5 min.

4. Remove and centrifuge for 10 min at 5200 x g.

5. Filter supernatant into screw top flask through Whatman # 1 filter paper.

6. Add 8 mL of ¼ Ringer's solution and vortex.

7. Centrifuge for 10 min at 2500 x g.

8. Filter through same filter paper. Rinse filter paper.

9. Remove pellet and place in new tube.

10. Wipe tube with ½ Kimwipe.

11. Place Kimwipe and filter paper into the 2nd tube.

12. Add 25 mL of 6N HCl to each tube (soluble and insoluble).

13. Autoclave tubes for 18 h at 121°C at 1.22 – 1.36 atm.

14. Remove and allow cooling to room temperature.

Preparation of hydroslyate

1. Add 1 g carbon decolorizing agent and vortex.
2. Filter samples into 500 mL volumetric flask for insoluble and 250 mL volumetric flask for soluble.
3. Rinse cap and original flask three times.
4. Rinse filter paper three times.
5. Dilute to desired volume.

Preparation and measurement of Hydroxyproline content

1. Place 1 mL diluted sample or standard in each tube.
2. Add 2 mL isopropyl alcohol.
3. Vortex
4. Pipette 1 mL oxidizing solution.
5. Vortex sample and let stand for at least 4 min.
6. Add 4 mL Erhlich's reagent and vortex.
7. Prepare each sample in duplicate.
8. Place tubes in 60°C waterbath for 25 min.
9. Place in cool waterbath for 5 min and vortex.
10. Read absorbance of samples and standards at 558 nm after zeroing with water.

Calculations

1. From absorbance standards, prepare a regression of µg/mL. Use regression to obtain µg/mL for each sampe.

2. Multiply µg/mL by the total volume to which the sample was diluted (250 for soluble and 500 for insoluble).

3. Divide by the grams of sample used (3.0 g).

4. Convert Hydroxyproline to collagen by multiplying the soluble by 7.52 and the insoluble by 7.25 to get µg collagen/g of sample.

5. Divide by 1000 to convert µg collagen to mg of collagen.

6. Report as soluble collagen, insoluble collagen, total collagen (soluble + insoluble) and % soluble (soluble/total*100).

Appendix K - Color Panel Descriptors

Initial Color
1 = Bleached, pale red
2 = Slightly cherry red
3 = Moderately light cherry red
4 = Cherry red
5 = Slightly dark red
6 = Moderately dark red
7 = Dark red
8 = Very dark red
Score to half-point increments

Display Color Score
1 = Very bright red
2 = Bright red
3 = Dull red
4 = Slightly dark red
5 = Slightly dark red or reddish tan
Borderline acceptability to panelist
6 = Moderately dark red to tannish red
7 = Tan to brown
Score to half-point increments

Discoloration Scale – Surface % MetMB
1 = None (0%)
2 = Slight discoloration (1-19%)
3 = Small discoloration (20-39%)
4 = Modest discoloration (40-59%)
5 = Moderate discoloration (60-79%)
6 = Extensive discoloration (80-99%)
7 = Total discoloration (100%)
Score to whole-point increments only

Appendix L - TBARS Procedures

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Adapted from:

Witte, V. C., G. J. Krause, and M. E. Bailey. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. J. Food Sci. 35:582-585.

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Solutions

9% Perchloric acid
- Prepare under fume hood
- Fill 2 L volumetric flask with ~1500 ml of distilled-deionized H_2O (**DD H_2O**)
- Slowly add 259 ml of 70% perchloric acid. Bring to volume with distilled-deionized H_2O
- Store refrigerated.

Distilled Water

0.02 M 2-Thiobarbituric acid (TBA)
- 1.4415 g 2-thiobarbituric acid / 500 ml
- Dilute to volume with DD H_2O, use magnetic stir bar to dissolve TBA
- Prepare immediately before use

Tetraethoxypropane (TEP) Stock Solution
- Take 0.44 g TEP to 100 ml volume with DDI H_2O [2×10^{-5} mol / ml]
- Pipette 0.5 into 500 ml volumetric flask, take volume with DD H_2O
- (This is the working stock solution) [2×10^{-8} mol / ml]
- Store refrigerated; bring to room temperature before use

TEP Standards
- Dilute the TEP working stock solution [2×10^{-8} mol / ml] by taking the following mls of to 50 ml in a volumetric flask with DD H_2O

mls Working TEP stock	solution Resulting [mol / 5 ml]
1	0.2×10^{-8}
2	0.4×10^{-8}
4	0.8×10^{-8}
5	1.0×10^{-8}
10	4.0×10^{-8}
30	6.0×10^{-8}
40	8.0×10^{-8}

Procedures

Sample preparation

1. Cut meat sample into small cubes, regardless if intact muscle or ground muscle.

2. Submerge cubed sample in liquid N_2 until liquid N_2 ceases boiling.

3. Chill blender cup by pouring a small amount of N_2 into blender cup.

4. Add frozen sample to blender cup; run blender for ~20 s to pulverize sample or until sample is completely pulverized / powdered.

5. Package labeled sample, removing as much air as possible.

6. Store sample at -80°C until ready to use.

Extraction and Absorbance Reading

1. Weigh 10 g of sample in duplicate into small blender cup.

2. Add 25 ml chilled DD H_2O to blender cup.

3. Add 15 ml chilled 9% perchloric acid to blender cup.

4. Blend for 15 s.

5. Pour contents of blender cup into filter-lined funnel (Whatman #2 filter paper).

6. Collect filtrate in 25 x 150 mm test tube.

7. Pipette 5 ml of filtrate from tube into 18 x 150 mm test tube.

8. Add 5 ml TBA solution to filtrate in the 18 x 150 mm test tube.

9. Vortex filtrate + TBA.

10. Store in the dark at room temperature for 24 h to allow color reaction to develop.

11. Develop standard curve using TEP Standards

    a. Use 0, 0.2×10^{-8}, 0.4×10^{-8}, 0.8×10^{-8}, 1.0×10^{-8}, 2.0×10^{-8} / ml TEP
    b. Pipette 5 ml of standard into 18 x 150 mm test tube; add 5 ml of TBA solution, vortex, and store as for test samples

12. Transfer developed solution to 13 x 100 mm test tube and measure spectral absorbance at 530 nm. Blank the spectrophotometer with a mixture of 5 ml DD H_2O and 5 ml of TBA solution.

13. Calculate TEP concentration in samples based on standard curve. Multiply by 0.72 to convert from TEP to TBA reactive substances (TBARS) values.

14. Report results as mg of malonaldehye / kg muscle tissue