

THE INFLUENCE OF GROWTH-PROMOTING TECHNOLOGIES ON THE BIOLOGICAL
STRUCTURES RESPONSIBLE FOR COOKED MEAT TENDERNESS

by

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B.S., Kansas State University, 2013

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2015

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Abstract

The objective of this body of work was to examine effects of growth-promoting technologies (GP) on *Longissimus lumborum* meat tenderness, focusing on alterations of muscle fiber cross-sectional area (CSA) and collagen solubility. Two studies were conducted and analyzed as randomized complete block designs with repeated measures with GP and day of postmortem aging (DOA) as main effects. Treatments consisted of: a control (CON), implant only (IMP), and implant and β -adrenergic agonist (COMBO). The β -adrenergic agonist utilized for the first was zilpaterol hydrochloride, while the second study examined ractopamine hydrochloride. Objective tenderness of strip loin steaks was measured through Warner-Bratzler shear force (WBSF) after 2 (study 2) or 3 (study 1), 7, 14, 21, or 35 d of postmortem aging. Muscle fiber CSA and collagen solubility were analyzed via immunohistochemistry and hydroxyproline content, respectively. For the first study there was a treatment \times DOA interaction ($P < 0.01$) for WBSF. Compared to CON steaks, IMP steaks had greater ($P = 0.01$) WBSF on d 3, but were similar ($P = 0.21$) by d 14. The COMBO steaks remained less tender at all-time points ($P < 0.04$) except d 21 ($P = 0.13$) when compared to the CON. Growth-promoting treatment increased the CSA of all three muscle fiber types ($P < 0.01$), but had no effect on collagen solubility measures ($P > 0.21$). The second study observed no treatment \times DOA interaction ($P = 0.54$) for WBSF, but GP increased ($P < 0.01$) WBSF across all DOA. Growth-promoting treatment tended to increase the CSA of type I and IIX fibers ($P < 0.10$), and increased ($P < 0.01$) type IIA fiber CSA. In agreement with the first study, there was no treatment \times DOA interaction or treatment effect on collagen solubility ($P > 0.75$). The addition of GP to feedlot heifer production increased WBSF of strip loin steaks and fiber CSA, but did not impact collagen characteristics.

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Acknowledgements

Wow, what a journey. If someone had said I would be pursuing my Master's degree when I started my undergraduate college degree, I would've said they were insane. However, this journey has been one of the most valuable and invigorating experiences that I have been blessed with throughout my life. There have been so many important people that have made this experience worthwhile and provided me with encouraging words when I needed it the most. First of all, I would like to thank the Gonzalez's, both John and Sara. Without Sara's trust, I never would've met John in the first place. It was her recommendation that allowed me to obtain my first position in the muscle biology laboratory as an Undergraduate Research Assistant. As I struggled through learning the techniques, Dr. John Gonzalez was there to guide me and make me more self-confident and efficient. Throughout my Master's program, Dr. Gonzalez served as my major professor and principle investigator for my research project. He provided me with support and criticism when needed to help make me a better scientist. Although my project will always be known as "the project from hell", it allowed me to understand the importance of collecting accurate data and how to properly follow a protocol! Thank you both for your positive words of encouragement and assistance over the past few years. I am glad I will always have your friendship to help guide me on my life journey. Also, I would like to thank my other committee members, Dr. Jim Drouillard and Dr. David Grieger. I appreciate the time you have spent serving on my committee and the support you have provided me through this process. The guidance of these four individuals has provided me with the tools that I will need to succeed in my first "big-kid" job and I can't express my gratitude enough.

Without the help of Kelsey Phelps, I probably would've quit grad school as soon as I started. She provided me with a shoulder to lean on when things started going wrong and helped

direct me in the right direction. Also, she helped me become more comfortable with the meat science portion of my project, because we all know how scary John Wolf can be when you first start graduate school. Thank you for putting up with my constant and annoying questions and always having words of encouragement when I start to doubt myself! I greatly appreciate all the things that you have assisted with for my project, including helping me understand SAS codes, troubleshooting the Hill method, helping me collect my samples at the plants, and anything else you did (which was a lot) that I have forgot to mention. I look forward to continuing the friendships I have made with you and Kendall (yes, even Seve). Once again, I don't know how I would've made it through graduate school if it hadn't been for your friendship and assistance.

Next, I would like to thank all of my laboratory mates that assisted me process my samples or understand my data. Mat Vaughn, Derris Burnett, and Jere Noel assisted me with several aspects of my project. From helping me weigh cattle to pipetting hundreds of samples into cuvettes, each one of you helped make my project much easier and for that I am extremely thankful. I would also like to thank the other graduate students that allowed me to assist with their projects and learn different animal research techniques. Julie Feldpausch was an excellent teacher for swine bleeding techniques and Cadra Van Bibber-Kreuger helped me become confident in moving cattle, working a squeeze chute, and bleeding techniques. Chad Paulk, thank you for helping me understand the statistics that you helped me with for my projects.

I would like to thank John Wolf for his assistance in the meat lab and for keeping me on my toes when it came to leaving data somewhere it shouldn't be! Also, a big thank you must go to the meat lab employees for showing me how to operate the big, scary equipment in the meat lab. I am confident enough to say that I can safely vacuum package and cut a strip loin into steaks without anyone else's help, and the meat lab employees had a lot to do with making that

possible. Another person that provided me with guidance was Sally Stroda, who assisted with the cooking part of my project in the sensory lab. She taught me the proper way to cook steaks and the importance of maintaining accuracy with sampling techniques prior to cooking. Also, I would like to thank the graduate students and feedlot workers that assisted with feeding the animals for my trials. This would not have been possible without the care you provide the animals and the hard work that each one of you puts into keeping the animals fed and happy. Also, a shout out to the minions (other undergraduate research assistants) that helped keep me from losing my mind and assisted with the collection of my data in the lab.

Finally, I would like to thank my Wichita family and friends for providing me the encouragement and love that I needed to finish this degree. My mom and dad always had time to take a phone call from my stressed out self and always were successful in calming me down. You two instilled the work ethic in me that I believe is my most valuable attribute, and for that I thank you! Mom, thank you for giving me the opportunity to develop this bond I have with animals by surrounding me with animals as you raised me. I could not have asked for a more rewarding field of work! Dad, thank you for always making me laugh and providing me a shoulder to lean on when times were difficult. If I needed a weekend to relax, I could always come home and relax with you whether we were fishing, playing cards, or drinking beer and playing pool. Christina, you were always there when I needed a laugh or even someone to talk to when I needed to vent to someone. Thank you for being my best friend! Rebekka, thank you for always being there for me if I needed some sisterly advice and for bringing my beautiful niece into this world! I wish you lived closer! Above all else, I would like to thank God for guiding me to this milestone in my life, and I hope to become a deeper, better Christian in the years to come.

Dedication

To my family for the love and support you have provided me and to the lifelong friends I
have made throughout this journey.

Chapter 1 - Introduction

As the cost to feed and produce cattle continues to rise, feedlot producers strive to improve efficiency of growth. According to the U.S. Department of Agriculture's National Agricultural Statistics Service, total cattle inventory as of January 1, 2015 was 89.8 million, which was up 2% from the previous year and is the first reported increase since 2007. Although the cattle population has struggled to increase until recently, total pounds of beef produced has remained constant due to advancements in production management and growth-promoting technologies (**GP**) employed during the stocker and finishing phases of production. Today, many producers utilize GP to improve the use of nutrients during production, which can result in greater carcass weights and yields. The two most common GP utilized by beef producers are anabolic implants and β -adrenergic agonists (**β -AA**). Duckett and Pratt (2014) reviewed benefits of implanting cattle and reported a \$102.62 advantage for cattle receiving at least one implant. When GP were removed from production, Capper and Hayes (2012) reported a 9.1% increase in the cost of production. Research demonstrates that combining the use of implants with β -AA can produce an additive response on efficiency measures and carcass traits when compared to programs that utilize the two technologies separately (Baxa et al., 2010; Woerner et al., 2011). Therefore, it could be hypothesized that economic benefit could be maximized by combining the two GP technologies.

Anabolic implants are available in several different products that contain synthetic compounds or naturally occurring hormones which produce a growth response and improve efficiency. Reinhardt and Wagner (2014) performed an extensive meta-analysis on the use of high-dose anabolic implants on growth and carcass traits. The study demonstrated the impact of combination implants containing trenbolone acetate (**TBA**) and estradiol (**E₂**) which can improve

ADG by 0.23 kg/d and HCW by 22.5 kg over non-implanted cattle. Unlike anabolic implants, the market currently only has two options for β -AA that are approved for use in finishing cattle. Ractopamine hydrochloride (**RH**), also known under the trade name Optaflexx (Elanco Animal Health, Greenfield, IN), is a β_1 -AA that has displayed improvements in ADG and feed efficiency, which in turn increases carcass weights and lean muscle yields (Bryant et al., 2010; Boler et al., 2012; Bohrer et al., 2014). An extensive meta-analysis reported a 8 kg increase in BW, improvements to ADG by 0.19 kg/d, and no impact on DMI (Lean et al., 2014). The other β -AA used in cattle production is zilpaterol hydrochloride (**ZH**). Known commercially as Zilmax (Merck Animal Health, Summit, NJ), this is a β_2 -AA that can have more pronounced impact on feed efficiency and carcass yields (Arp et al., 2013; Brown et al., 2014). Lean et al. (2014) reported ZH can increase BW by 8 kg, ADG by 0.15 kg/d, and HCW by 15 kg. The prominent effects of these GP on feedlot performance and carcass composition has been a popular research topic in recent years, but the negative impact on tenderness that is associated with use of GP can have undesirable results on consumer satisfaction.

Meat tenderness is a major determinant of consumer satisfaction and can be impacted by several factors (Lusk et al., 2001; Dikeman, 2007). Pre-harvest factors that influence beef tenderness include animal characteristics, such as sex, genetics, or production techniques such as diet, time on feed, and the use of GP (NCBA, 2007). When analyzing the sex effect, Choat et al. (2006) reported decreased WBSF measures for steaks from steers when compared to the heifers at a common marbling score. Breeds with a greater proportion of Angus genetics had improved tenderness measures when compared to those with decreased amounts of Angus genetics (Stolowski et al., 2006). Several meta-analyses have demonstrated the negative impact of GP on meat tenderness (Duckett and Pratt, 2014; Lean et al., 2014). The magnitude of the GP effect on

meat quality traits is impacted by several factors, including type, potency, and duration of growth-promoting technologies that are utilized during growth (Arp et al., 2013). Duckett and Pratt (2014) performed an extensive meta-analysis and reported an increase in Warner-Bratzler shear force (**WBSF**) by 0.1 kg for steaks from cattle subjected to implants. Lean et al. (2014) reported an average increase in WBSF of 0.8 and 0.2 kg for steaks from cattle subjected to ZH and RH, respectively. Also, previous research has observed the negative impact of implants and β -AA on quality grade (Garber et al, 1990; Montgomery et al., 2009a; Culp et al., 2013), marbling score (Faulkner et al., 1989; Winterholler et al., 2008; Holmer et al., 2009a), and water holding capacity (Rathmann et al., 2009). These measures provide some explanation for tenderness changes between meat products. Therefore, the objectives of this literature review are to: 1) examine impact of growth-promoting technologies on skeletal muscle characteristics of *Longissimus lumborum* muscle, 2) examine impact of GP on tenderness of *Longissimus lumborum* steaks, and 3) to correlate the meat tenderness to skeletal muscle characteristics.

Chapter 2 - Review of Literature

Growth-Promoting Technologies Impact on Growth Performance and Carcass Characteristics

Implants

Anabolic implants in beef production systems have been studied extensively since the approval for use by the Federal Drug Administration in the 1950's (FDA, 2014). Several products are currently available for use in the United States, each consisting of various compounds and concentrations for use at different stages of animal growth. Approximately 97% of cattle entering the feedlot have received at least one anabolic implant during growth (NAHMS, 2000). Implant strategies are employed to improve animal efficiency and carcass yields. Producers consider the proper implant strategy for a specific cattle type based upon the active ingredients and potency of the implant. Products that are available for use in beef cattle production consist of: Synovex (**SYN**)-C, -S, -H, Choice, or Plus (Zoetis, Florham Park, NJ) which contains progesterone, E₂, and/or TBA at various concentrations for stocker or feedlot cattle; Ralgro (Merck Animal Health) which consists of zeranol, a synthetic estrogen compound; Revalor (**REV**)-200, -G, -H, -IH, -IS, -S, -XS (Merck Animal Health) which consists of different levels of TBA and E₂; and Component TE-G, TE-H, TE-IH, TE-S, TE-IS, T-H, TE-200 (Elanco Animal Health), which also consists of combinations of TBA and E₂ (Parish and Rhinehart, 2011). These compounds function to increase net protein accretion through an increases in protein synthesis and nitrogen retention (Cecava and Hancock, 1994). Much of the research focuses on effects of these different implant programs on cattle performance, carcass measures, and circulating body protein or hormone levels.

Feedlot Performance and Carcass Effects

As introduced above, several products are available for producers to utilize during the stocker and feedlot phases of cattle growth. The different compounds include: estradiol, estradiol benzoate (**EB**), progesterone, TBA, and testosterone propionate (**TP**). The use of these compounds can improve efficiency and gains in feedlot cattle (Borger et al., 1973; Cole et al., 1984; Faulkner et al., 1989; Moran et al., 1991; Rumsey et al., 1992a; Rumsey et al., 1992b). The different products available range in potency and are recommended for different stages during growth. The aggressiveness of an implant is dependent upon the compounds used, concentration of each component, and can be considered as high or low potency. A terminal implant will be more potent and are beneficial when administered at the end of growth and when cattle are on a high energy diet. These implants can be employed at the end of the growth phase to maximize the response in efficiency and carcass yields. The following section will examine the impact of these compounds for a single or reimplant or delayed implant programs on animal performance and carcass characteristics.

Single Implant Programs

Depending on the status of the animal, cattle may only be implanted once during the feedlot phase. This single implant program can display improvements in growth and provide producers with a strategy for improving profitability. The type of implant can be categorized as estrogenic, androgenic, or a combination of the two different compounds. Estrogenic compounds include progesterone, E₂, EB, and zeranol, a synthetic estrogen compound. Rumsey (1982) examined the effect of SYN-S (200 mg progesterone and 20 mg EB) implants on steer performance. Steers administered an SYN-S implant had increased ADG and tended to have increased DMI and gain to feed (**G:F**) when compared to non-implanted controls. Cole et al. (1984) observed the effect of zeranol implants on yearling Angus steer performance. Daily gain

was measured across 143 d of feeding and the effect of the zeranol implant was observed. Angus steers that had received an implant had improved daily gain of approximately 16% on d 14, 28, and 143 of feeding. Hopkins and Dikeman (1987) observed the effect of E₂ on performance of steers and bulls for 200 d intervals from birth to slaughter. The implanted steers and bulls did not have increased final BW following treatment with E₂ implants. Interestingly, steers that received an implant had decreased ADG and feed to gain (F:G) by 10% when compared to the control bulls. The implanted bulls did not differ from the control bulls for ADG and F:G, but had decreased F:G by 12% when compared to the implanted steers. This study demonstrates the importance of the hormone concentration utilized in an implant which must meet the body's requirements if an animal has had the natural source of hormones removed via castration or spaying.

Borger et al. (1973) examined the effect of zeranol implants administered on d 1 and 84 of the feedlot phase of finishing steers on growth performance. Steers that had received an implant had 14.9 kg heavier final BW and had 7.8% greater ADG when compared to the non-implanted controls. In this study, the ADG was increased by 8.5% on d 84 and this percentage remained constant due to the second implant of zeranol on d 84 of the finishing period. Lemieux et al. (1990) observed the effect of estrogenic implants containing zeranol or 200 mg progesterone and 20 mg EB on crossbred yearling steer production. The authors reported no difference between treatments for ADG, DMI, and F:G measures due to implantation. In contrast, Rumsey et al. (1992b) reported increased ADG of 11.6% for steers implanted with a similar product containing 200 mg progesterone and 20 mg EB (EP) when compared to non-implanted steers. For a second set of steers, Rumsey et al. (1992b) reported a 21.5% increase in ADG for implanted steers when compared to the non-implanted counterparts. In another study

reported by Rumsey et al. (1996a), the authors observed the same implant product in a different set of steers. Similar to their previous findings, the authors reported an increase of 12.5% in ADG due to implant treatment. Rumsey et al. (1996b) reported an increase in ADG of 29% for another group of steers implanted with the same product as the previous two studies. In a later study, Rumsey et al. (1999) observed the effect of SYN-S implants in both crossbred steers and heifers. With both sexes combined, the implanted cattle had increased final BW of 31 kg, ADG by 11%, decreased DMI by 7% and tended to have improved G:F. Mader (1994) evaluated the effect of single or double zeranol implants administered during the finishing period on feedlot steer performance. For the steers that received two zeranol implants, final BW and ADG were increased when compared to the single implanted and control steers. Dry matter intake and G:F were increased for double implanted steers when compared to control steers, but did not differ from the single implanted steers. Also, the single implanted steers had increased final BW and ADG, but did not differ for DMI and G:F when compared to control steers. Kreikemeier and Mader (2004) reported increased final BW by 9.1 kg, ADG and G:F by 6% following 105 d feeding period for heifers that received an implant containing 24 mg of E₂. Pampusch et al. (2008) examined the effect of E₂ implants on steer BW in the days following the administration of the implant. The total BW of steers 14 d after implanting was similar to steer that did not receive an implant; however, the implanted steers had increased BW by 15 kg after 28 d being implanted when compared to controls. Also, the DMI was not different during this period for implant and non-implanted steers. The addition of estrogenic implants to feedlot steer and heifer production can improve efficiency and final BW as demonstrated by the previous studies.

Androgenic compounds that are available for use in cattle production include TP and TBA, which can improve growth efficiency when employed during the feedlot phase. Crouse et

al. (1987) observed the effect of TBA on feedlot performance of intact or ovariectomized heifers. The authors reported no difference in performance results for both types of heifers when implanted with a TBA implant. Faulkner et al. (1989) observed the effect of TP implants on cull cow performance. For this study, no advantage was achieved during the realimentation period when cull cows were implanted with TP. Apple et al. (1991) observed the effect of TBA implants administered during the feeding period on Holstein steer performance. Throughout the entire feeding period, the non-implanted steers had similar ADG as the steers only implanted with TBA. Hunt et al. (1991) observed similar results as the previous study, as steers and bulls implanted with 120 mg TBA did not perform differently than non-implanted controls. Hayden et al. (1992) compared the effect of TBA implant to non-implanted steers and observed no difference for BW and ADG across the 80 d duration of the study. Similarly, Kreikemeier and Mader (2004) reported similar final BW, ADG, and G:F for heifers that received a TBA implant when compared to their control counterparts. Pampusch et al. (2008) observed increased total BW during growth for steers implanted with TBA 14 d prior to the measurement, while DMI did not differ between the treatments.

A more effective choice of implant is a combination implant that consists of both an estrogenic and androgenic compound which can further improve efficiency measures during the feedlot phase. Ray et al. (1969) examined the effect of SYN-H (200 mg TP and 20 mg EB) for yearling heifers in two separate studies. For implanted heifers, the ADG reported was 3% and 14% greater than the control heifers in experiment 1 and 2, respectively. DeHaan et al. (1990) observed the effect of an implant containing 200 mg TP and 20 mg EB that was administered on d 1 and 85 of the feedlot heifer performance. The implanted steers had increased DMI by 8% and improved G:F by 7%, while ADG was not impacted. Garber et al. (1990) observed the effect of

SYN H or S (200 mg TP and 20 mg EB; 200 mg progesterone and 20 mg EB) implants on spayed and intact heifers. There was a tendency for an interaction between spaying and implantation in this study. For intact and spayed heifers, the use of an implant increased the ADG by 8 and 26% and F:G by 5 and 17%, respectively. This study demonstrated the need of implanting spayed heifers to replace the removed hormones, as spaying and not implanting decreased ADG. Cranwell et al. (1996) observed the effect of a combination implant in mature beef cows. Cull cows that received a combination implant had increased final BW by 26 kg, total weight gain by 26 kg, and G:F by 22% when compared to the non-implanted cows. The improvement to performance measures demonstrates the ability of implants to improve growth performance of cull cows during the realimentation phase. Henricks et al. (1997) did not observe improvements to ADG and G:F for heifers that received an implant containing 140 mg TBA and 14 mg E₂. In contrast, Popp et al. (1997) observed the effect of TBA and E₂ implants on heifer growth performance in the final period of the feedlot phase. The heifers that received an implant had heavier final BW, improved ADG, and feed efficiency compared to the non-implanted controls. Similarly, Cook et al. (2000) observed increased final BW by 19.2kg, ADG by 13%, and feed efficiency by 11% for heifers receiving an implant containing 140 mg TBA and 14 mg E₂. Dunn et al. (2003) observed the impact of REV-S implants on ADG of feedlot steers. When compared to the non-implanted steers, the REV-S implant increased ADG by 34% during the finishing period. Kreikemeier and Mader (2004) observed the effect of implantation with a combination implant on performance measures of crossbred Angus heifers. Heifers that received a combination implant had improved ADG by 12.6% and G:F by 12% over the 105 d feeding period when compared to the non-implanted heifers. Neill et al. (2009) reported increased BW gain of 22.8 and 3.7 kg, respectively, for cull cows implanted with a REV-200 implant for the

first 36 d and the last 34 d of feeding, but over the total feeding period there was no difference for BW gain between the non-implanted and implanted cows. For feed efficiency, the implanted cows had improved G:F by 33% during the first 36 d on feed, but did not differ for the remainder of the feeding period or over the total feeding trial when compared to the controls.

The effect of combination implants on bull and steer performance has been studied extensively. Boucque et al. (1988) examined the effect of anabolic treatment on bull performance. Bulls that received the anabolic treatment were injected with a cocktail of androgens and estrogens and had increased ADG of 0.18 kg when compared to no injected bulls. Perry et al. (1991) observed the effect of an implant containing 140 mg TBA and 28 mg E₂ in finishing steers of 3 different breed types. When comparing implanted steers to non-implanted steers, final BW and ADG were increased, while days on feed and DMI were decreased for implanted steers. Apple et al. (1991) observed increased ADG for steers that received an implant containing TBA, progesterone, and EB when compared to non-implanted steers. Other measures of DMI and F:G did not differ when the entire feeding period was observed.

Hunt et al. (1991) examined the effect of two implants containing 120 mg TBA and 24 mg E₂ given at the same time during the finishing phase for steers and bulls. When compared to the controls, steers that received the implants had similar ADG and F:G, while DMI was increased. Bulls performed similar with or without an implant during the finishing stage of growth. In contrast, Cecava and Hancock (1994) reported increased final BW of 5% and ADG of 18% for steers implanted with a combination of estrogenic and androgenic implants. Gerken et al. (1995) utilized cloned steers to determine the effects of REV-S (120 mg TBA and 24 mg E₂) implants on animal performance. For the steers that were implanted, the ADG reported was 38.1% greater and G:F was 29.6% improved when compared to controls. The ratio of EB to TBA

in a single implant was examined in both steers and heifers (Herschler et al., 1995). A 1:10 ratio implant increased the rate of gain and improved feed conversion of both steers and heifers when compared to an implant containing a ratio of 1:5. Regardless of the ratio used, ADG was improved when an implant was administered than when steers and heifers were not implanted. Foutz et al. (1997) observed the effect of SYN-S or REV (140 mg TBA and 20 mg E₂) on steer performance. Steers that received the REV implant had increased final BW, ADG, and F:G when compared to control and SYN-S implanted steers over the entire feeding period. For the steers that received the SYN-S implant, the reported DMI was decreased when compared to controls and the REV implanted steers. Hermesmeier et al. (2000) examined the effect of REV-S (120 mg TBA and 24 mg E₂) or SYN-Plus (200 mg TBA and 28 mg E₂) implants on growth performance in crossbred steers. Regardless of the type of implant used, implanted steers had greater gains and improved feed efficiency when compared to their non-implanted counterparts. Comerford et al. (2001) examined the effect of REV-S implants during finishing for Holstein steers fed for different amounts of days on feed. After 56 d on feed, steers that had received an implant had 8% increased ADG and 3% decreased DMI when compared to non-implanted steers. Following 196 d on feed, the implanted steers had increased ADG by 3%, G:F by 4%, and decreased DMI when compared to the controls. As the feeding period continued, the effect of the implant decreased which was displayed by the decrease in performance displayed by the implanted steers after 196 d on feed. Pampusch et al. (2003) analyzed the effect of a REV-S implant on yearling steer performance. When compared to the non-implanted controls, steers that had received an implant had improved ADG by 36% and G:F by 34%, which is significantly greater than the previous study. The previous study analyzed the effect of the REV-S implant across a 196 d feeding period while the current study observed the effect during the first 26 d of

implantation. The greater response observed in the current study can be attributed to the efficacy of the implant during the initial period after implanting when compared to the entire feeding period following implantation. In contrast to the previous study, the DMI for implanted steers was not different than non-implanted steers. Also, Bruns et al. (2005) observed improvements in BW, ADG, and G:F for feedlot steers receiving a combination implant. Winterholler et al. (2008) reported that a conventional management system, which utilized a terminal implant, increased final BW by 26 kg, ADG by 14.5%, and G:F by 7% when compared to a natural system that did not employ an implant during finishing. In a second study by the same authors, final BW, ADG, and G:F were also increased by 6 kg, 7.8 and 5.9%, respectively, as a result of implanting in steers. Smith et al. (2007) examined the effect of SYN-Plus implants administered twice during the finishing period on feedlot steer performance. Across the 133 d feeding period, implanted steers had increased final BW by 27 kg and improved ADG by 15% when compared to the nonimplanted steers. Pampusch et al. (2008) reported increased total BW for steers implanted 28 d before BW measurement which were similar in weigh prior to implantation. The effect of three different combination implant strategies was examined for Holstein steer performance by Bass et al. (2009). An implant containing one of the following combinations: EP, 80 mg TBA and 16 mg E₂ (**MT**), or 120 mg TBA and 24 mg E₂ (**HT**) was administered to steers 104 or 131 d prior to harvest. All three implant strategies increased final BW by 18.2 kg and ADG by 13% when compared to steers that did not receive an implant, and performed similar to one another during the finishing period. Baxa et al. (2010) observed the effect of REV-S implants on steer feedlot performance. The steers that received an implant had increased final BW, ADG, and G:F compared to the non-implanted steers. Carter et al. (2010) reported a tendency for improved ADG for implanted steers when compared to non-implanted steers. Parr et al. (2011) examined

the effect of a single REV-S or REV-XS (200 mg TBA and 40 mg E₂) on steer feedlot performance. During the entire feeding period, steers that received an implant had increased final BW by 48 kg, ADG by 19.6%, G:F by 2.4%, and DMI by 5.9% when compared to the no implant steers. When comparing the two different implant strategies, REV-XS steers had increased final BW by 18 kg, ADG by 5.6%, and G:F by 4.5%, but DMI did not differ over the entire finishing period. This study demonstrated the ability of a greater TBA and E₂ dose to improve the efficiency of steers more than an implant containing a lesser amount of TBA and E₂. Cleale et al. (2012) administered combination implants containing the greater dose of 200 mg TBA and 28 mg E₂ to feedlot steers. When compared to the controls, the steers receiving a combination implant had increased ADG by 13.4%, DMI by 4%, and G:F by 9%. When compared to the previous study, the magnitude of increase in ADG was 7.8% greater for the steers in the current study. Cleale et al. (2013) performed another study utilizing 100 mg TBA and 14 mg E₂ during feedlot heifers. When compared to the non-implanted heifers, the TBA and E₂ implant increased the final BW by 15 kg, ADG by 7%, and G:F by 6% during the finishing phase of feedlot heifers. The previous studies demonstrate the ability of combination implants to improve growth performance measures.

Due to the feedlot performance improvements, the effect of anabolic implants on carcass characteristics was observed for feedlot heifers. Crouse et al. (1987) examined the effect of TBA implants on performance of heifers. Implanted heifers had similar final BW, but an increased LM area by 4.1 cm² when compared to controls. Adams et al. (1990) observed increased HCW by 13.1 kg for heifers implanted with SYN-H, but displayed no impact of implantation on dressing percentage (**DP**) and LM area. Garber et al. (1990) observed no implant effect on HCW, DP, and yield grade (**YG**) measures, but implanted heifers had an increased LM area by 7.5 cm² when

compared to the non-implanted controls. Moran et al. (1991) examined the effect of TBA implants in crossbred heifers on the improvements of carcass characteristics. Through improvements to gains during the feedlot phase, HCW was increased by 12 kg for TBA implanted heifers when compared to the non-implanted heifers. When utilized in mature beef cows, implants produced heavier HCW, increased LM area, and improved YG (Cranwell et al., 1996). Herschler et al. (1995) reported increased HCW and LM area for implanted steers when compared to non-implanted steers. In contrast, Henricks et al. (1997) observed no difference in HCW, DP, or LM area for heifers that were implanted or not implanted with a combination or TBA only implant. Cook et al. (2000) reported 12.7 kg increased HCW and 2.7 cm² larger LM area for implanted heifers. Kreikemeier and Mader (2004) displayed an increase of 11.9 kg in HCW, but no other carcass measures were influenced by combination implants in crossbred heifer production. Neill et al. (2009) did not display differences for HCW, DP, LM area, and YG for cows implanted versus cows not implanted. In contrast, Cleale et al. (2013) observed increased HCW of 12.1 kg and LM area of 3.8 cm² for heifers that received a combination implant.

Borger et al. (1973) utilized an implant containing zeranol that was implanted twice during the finishing phase for steers. The carcass traits observed were HCW and LM area which were not affected by implanting for this study. Rumsey et al. (1982) displayed a tendency for DP to be increased due to SYN-S implants during steer finishing production. The authors also reported no difference in LM area for implanted and non-implanted steers. Hopkins and Dikeman (1987) reported similar HCW and DP for implanted steers and bulls and the controls. Other carcasses measures were impacted differently due to implants. The bulls and steers that received an E₂ implant had decreased LM area by 12.5 cm² when compared to control bulls.

Yield grade for implanted steers was increased as a result of E₂ implants when compared to the implanted and control bulls. Perry et al. (1991) reported increased HCW by 12 kg for implanted steers, but no difference in DP and LM area measures. In contrast, Hunt et al. (1991) observed no difference in HCW, LM area, and YG for implanted and control steers. For the same study, bulls implanted with a combination implant had 13 kg increase in HCW, but similar LM area and YG when compared to control bulls. Cecava and Hancock (1994) reported increased HCW of 21 kg and LM area of 4.8 cm² for steers implanted with a combination implant. In contrast, Mader (1994) observed no difference in DP, LM area, or YG for implanted or non-implanted steers. Gerken et al. (1995) reported increased final BW of 42 kg and HCW of 32 kg for steers that received an implant containing 120 mg TBA and 24 mg E₂. Herschler et al. (1995) reported heavier HCW by approximately 8 kg and larger LM area by approximately 3 cm² for steers implanted with a 1:10 ratio of E₂ and TBA when compared to steers implanted with a ratio of 1:5. Foutz et al. (1997) evaluated the effects of an estrogenic implant alone or in combination with TBA on carcass characteristics of crossbred yearling steers. Steers that were administered an implant, regardless of type, had heavier HCW and improved YG. Hermesmeier et al. (2000) observed increased HCW by 4 kg, DP by 0.4% and LM area by 2 cm² for implanted steers when compared to non-implanted controls. Bruns et al. (2005) and Boles et al. (2009) reported increased HCW up to 22.5 kg and larger LM area up to 4.7 cm² for implanted steers when compared to the non-implanted steers. Winterholler et al. (2008) also observed increased HCW by 17 kg and decreased YG by 12% for steers that were assigned to a conventional management system and received an implant during finishing when compared to a natural system. A second study reported by the same authors had similar results in HCW as a result of implanting steers. Kellermeier et al. (2009) reported increased LM area by 8.6 cm² and decreased YG by 18.3% for

steers that received a REV-S implant. Bass et al. (2009) observed increased HCW by 11.4 kg and LM area by 5.2 cm for steers receiving three different implant strategies discussed previously above. The three implant strategies performed similar for HCW; however, the steers that received MT and HT implants had increased LM area by 2.5 and 3.2 cm² when compared to the steers that received an EP implant, respectively. Baxa et al. (2010) reported increased HCW by approximately 10 kg, DP by approximately 1%, and LM area by approximately 5 cm² for steers that received a REV-S implant when compared to the non-implanted steers. The studies introduced above state that anabolic implants administered to cattle in the feedlot phase can create additional gains and increase lean muscle yields.

With changes to the metabolism of nutrients provided to the animal, quality traits can be impacted in final meat products. Implants containing both estrogenic and androgenic compounds and administered later in the growth curve have a greater impact on adipose deposition. Crouse et al. (1987) reported similar marbling scores and 12th-rib fat thickness (FT) for TBA implanted and non-implanted heifers. Garber et al. (1990) detected decreased marbling scores and quality grade for crossbred heifers receiving SYN-H (200 mg TP and 20 mg EB) implants. In contrast, Adams et al. (1990) displayed no effect on marbling or QG due to SYN-H implantation for crossbred heifers, but observed a reduced proportion of fat within the *Longissimus* muscle. Other authors also reported no difference in marbling score for cattle receiving testosterone and E₂ implant (DeHann et al., 1990; Hunt et al., 1991). Mader (1994) reported no difference in FT, but implanted steers had decreased marbling scores when compared to the non-implanted controls. Herschler et al. (1995) examined the effect of a TBA and E₂ implant and reported no difference for marbling score or FT for feedlot heifers. Cook et al. (2000) observed no impact on 12th-rib FT or marbling score for feedlot heifers that did or did not receive an implant containing 140 mg

TBA and 14 mg EB. Similarly, Kreikemeier and Mader (2004) reported no difference for marbling score between controls and heifers that received an implant containing only E₂ or TBA. In contrast, when implanted with a combination implant, heifers had decreased marbling scores by 7.6%, but no difference in 12th-rib FT. Neill et al. (2009) observed no difference in 12th-rib FT or marbling score for cull cows administered a combination implant. Smith et al. (2007) examined the effect of SYN-Plus implants on heifer adipose measurements. The heifers implanted had similar FT and marbling score when compared to the non-implanted heifers. Cleale et al. (2012) displayed decreased marbling scores, but similar 12th-rib FT for heifers that received a combination implant. When examining the quality grade distribution, the amount of choice graded carcasses was decreased by 7.5%, while the select carcasses were increased by 9.8% when an implant was administered during finishing. Cleale et al. (2013) examined the effect of E₂, TBA, or TBA and E₂ implants on heifer quality traits. For this study, the E₂ and TBA implanted heifers had similar marbling score when compared to the controls and each other, while the TBA and E₂ implanted heifers had decreased marbling scores when compared to the other three treatments. In contrast, there was no implant effect on 12th-rib FT or quality grade distribution for this study. The impact of implants on quality grade of heifer carcasses is inconsistent, but decreased marbling score, quality grade, and FT have been reported due to employing an anabolic implant during the finishing period.

The use of anabolic implants during steer feedlot production can have detrimental impacts on adipose measurements within the carcass. Rumsey et al. (1982) reported no difference for marbling score, 12th-rib FT, and quality grade when comparing non-implanted and implanted steers. DeHaan et al. (1990) also reported no difference between implanted and non-implanted heifers when observing marbling score and FT. Perry et al. (1991) observed no difference in

marbling or 12th-rib FT for implanted steers when compared to non-implanted steers. Similarly, Apple et al. (1991) observed the effect of TBA and E₂ in Holstein steers. For this study, marbling scores and quality grade were unaffected by implantation. Dalke et al. (1992) utilized crossbred steers and observed similar results to the previous study for carcass quality characteristics, but displayed a decreased fat content in the LM. Cecava and Hancock (1994) observed no implant effect on marbling score, 12th-rib FT or quality grade for steers receiving a combination implant. Also, Gerken et al. (1995) observed no implant effect on marbling score and 12th-rib FT steers that received three different types of implant treatments. Foutz et al. (1997) and Henricks et al. (1997) also reported no change to marbling score or 12th-rib FT for steers receiving a single implant. Hermesmeier et al. (2000) reported an intake level × implant type interaction for marbling score of steers implanted with a REV-S or SYN-Plus and fed a restricted or ad libitum diet. The steers that received a restricted diet and SYN-Plus implant and the ad libitum diet and REV-S implant had the lowest marbling scores, while the ad libitum intake control steers reported the greatest marbling score for this study. When steers were fed to a common endpoint, implanting did not impact 12th-rib FT for steers fed restricted and ad libitum diets. Smith et al. (2007) and Winterholler et al. (2008) reported no difference in 12th-rib FT or marbling score for steers administered a combination implant. Parr et al. (2011) also displayed no difference in 12th-rib FT, marbling score, or quality grade distribution for steers administered a REV-S implant or not implanted. In contrast, Kellermeier et al. (2009) observed decreased 12th-rib FT by 0.29 cm and marbling score by 6 units for steers implanted with a REV-S implant. Similarly, Bass et al. (2009) reported decreased 12th-rib FT for Holstein steers that receive a MT implant, while the steers implanted with the more potent HT implant did not differ from the less potent implanted steers or the control steers. Marbling score was also decreased for the EP and MT implanted

steers when compared to controls and HT implanted steers. Also, the quality grade for implanted steers was decreased and in the mid-select range, while non-implanted steers were reported in the low choice category. Baxa et al. (2010) observed no difference in 12th-rib FT, but reported decreased marbling scores by 4.4% for steers that received a REV-S implant 91 d prior to slaughter. Igo et al. (2011) reported decreased marbling scores for cattle that did not receive a TBA and E₂ implant. Cleale et al. (2012) detected decreased marbling score for SYN-Plus implanted steers when compared to the non-implanted controls. In the same study, the authors did not display any differences in the amount of 12th-rib FT between the two treatments. When observing quality grade distribution, the implanted steers had an 11.8% decrease choice and an 11.5% increased select graded carcasses when compared to the controls. Despite the inconsistent results of implantation on quality scores, the use of implants during production has the potential to cause decreased fat deposition and result in lower quality carcasses.

Re-Implant Strategies

Because improvements were displayed by one implant programs, the effect of implant timing and re-implanting in finishing cattle has been examined to determine an appropriate implant strategy. Implants containing the selected hormones are released slowly over time, and the lifespan of a pellet-type implant is typically 100 d, whereas the growth phase of cattle can last longer than 365 d. As an animal ages, the lean growth potential begins to slow down, so the margin of response can be increased if anabolic implants are utilized when the animal is reaching the end of the growth curve (Moran et al., 1991). Simms et al. (1988) examined the effect of sequential implanting of zeranol during different phases of production on steer growth performance. When assigned, the second implant was administered to steers after 56 d on feed. During the finishing phase, steers that received two zeranol implants had improved ADG by 4 or

6.5% when compared to the steers that received only one implant or no implants, respectively. Final BW was increased by 25 kg for the steers that received two finishing implants when compared to controls. The steers that received only one implant during finishing had similar final BW to control and the twice implanted steers. This study demonstrated the ability of re-implanting to positively impact the efficiency and weight of steers during the finishing phase. Scheffler et al. (2003) observed the effect of implant strategy on growth performance of Holstein steers. Component TE-S implants were utilized and administered to steers assigned to an implant treatment on once on d 224, twice on d 112 and 224, or three times on d 0, 112, and 224. The steers that received an implant on d 0 had the greatest ADG from d 0 to 112; after which, they had similar ADG to the twice implanted steers for the rest of the finishing period. The steers implanted once on d 224 had similar ADG to the other implanted steers for the rest of the finishing period. Across the entire feeding period, the steers that were implanted at least two times had increased ADG, while the steers implanted once had similar ADG when compared to the control steers. Dry matter intake was increased for the steers implanted three times, while the other treatments had similar DMI. For G:F, the once and twice implanted steers had improved the efficiency of growth when compared to the control steers, but the steers implanted three times had similar G:F when compared to the control and other implant treatments.

Bryant et al. (2010) examined the effect of reimplanting or administering a single implant during finishing period of feedlot steers. Prior to reimplanting, the BW, ADG, and G:F were similar for the two implant treatments, which were also greater than the non-implanted control steers. From the reimplant period to the end of feeding, the reimplanted steers had 10% greater ADG and 8% more efficient than the steers that received only one implant. At the end of the feeding period, the reimplanted steers were 16.6 and 56 kg heavier than the single implanted

and non-implanted steers. Also, the ADG and G:F were improved by 6% and 4%, but DMI was not different for the reimplanted steers compared to the single implanted steers. Woerner et al. (2011) examined the effect of a reimplant program on steer feedlot performance. The steers were either initially implanted with an implant containing 80 mg TBA and 16 mg E₂ followed by a terminal implant containing 120 mg TBA and 24 mg E₂ on d 63 or implanted with a terminal implant only on d 63 of the feeding period. The BW and ADG of steers following initial 63 d feed period was increased by 12 kg and 10%, respectively, for the steers that received an initial implant. In contrast, when the entire feeding period was combined the two implant treatments did not differ in final BW, but reimplanted steers had 4% increased ADG. Parr et al. (2011) observed the effect of an initial only implant program or the addition of another implant later on in the feeding period. The steers assigned to receive an implant were administered an implant containing 80 mg TBA and 16 mg E₂ followed by a REV-S implant or administered a single REV-S implant at processing. For feedlot performance measures, the steers implanted twice had similar final BW, ADG, and DMI, but the G:F was improved by 3% when compared to the single implanted steers. In a second experiment, Parr et al. (2011) reported no difference in final BW, ADG, DMI, or G:F for steers implanted once or twice during finishing. Loy et al. (1988) examined the effect of zeranol or SYN-S implants administered on d 1 only or re-implanted on d 84 of finishing on crossbred steer performance. Regardless of implant treatment, performance measures of ADG, weight adjusted DMI, and non-adjusted G:F were increased 8.1, 3.7, and 7.4%, respectively, when compared to the non-implanted controls. When the two implant treatments were compared to each other, the ADG was greater for SYN-S implanted steers. Also, the steers implanted twice did not perform differently than the steers only implanted with one implant regardless of the implant compound used. Rumsey et al. (1992b) examined the effect of

a reimplant program with SYN-S d 0 and 60 on steer performance for two different trials. For the first trial, the reimplanted steers did not differ in ADG and DMI when compared to the control steers. When compared to the single implanted steers, the reimplanted steers had decreased ADG by 12.5%, while DMI was not different. The second trial displayed greater ADG and decreased DMI for the reimplanted steers when compared to the control group. When the two trials were combined, the reimplanted program had improved ADG by 22% and had decreased DMI by 15.7% when compared to the control steers.

Re-implanting cattle allows for greater potential of growth for feedlot heifers when compared to those who only are implanted once throughout their life (Rumsey et al., 1992b; Scheffler et al., 2003). Faulkner et al. (1989) evaluated the effect of SYN-H or TP on feedlot performance measures of crossbred heifers. The heifers received one of the following four treatments: 1) control, no implant administered; 2) SYN-H implant on d 1 and 84; 3) a TP implant on d 1 and 84 (**LT**); or 4) a TP on d 1 (**HT**). The HT heifers had improved ADG and F:G at d 157 of feeding when compared to the other three treatments. Samber et al. (1996) examined the use of 7 different implant strategies on animal performance. Regardless of implant strategy, cattle that received an implant had increased ADG up to 17% and G:F up to 11% for steers administered 3 implants containing TBA and E₂. The efficiency of growth was improved for steers receiving at least two implants containing TBA and E₂ during finishing. Also, the steers that received an initial implant performed similar for ADG and G:F when compared to the steers that were implanted 30 d later. DeHaan et al. (1990) observed the effect of an implant containing 200 mg TP and 20 mg EB administered on d 1 and 85 on feedlot heifer performance. The heifers that received an implant had improved ADG by 14% and G:F by 9%. Moran et al. (1991) studied the impact of TBA, E₂, or TBA and E₂ implants administered once or multiple times across

finishing on heifer performance. Across the entire feeding period, heifers that received multiple implants of TBA, E₂, and zeranol had improved ADG. The authors reported no difference between the heifers that received an implant once and multiple times over the finishing period. Mader and Lechtenberg (2000) examined the effect of a terminal implant program with or without reimplanting on heifer performance. Measures of final BW and ADG were increased for heifers receiving two implants when compared to heifers that on received one implant during finishing. Dry matter intake tended to be increased for reimplanted heifers, but G:F was not different between the treatments. Sissom et al. (2007) studied the effect of a less potent combination implant at the beginning of the study followed by another implant of TBA or implant initially with a more potent implant on feedlot heifer performance. There was no implant effect on heifer performance measures of ADG, DMI, and G:F when subjected to two different implant strategies. Despite the inconsistencies, the use of multiple combination implants can improve the efficiency of beef cattle during the feedlot phase of production.

As animal performance is improved with the administration of two or more implants during finishing the carcass yields also have the potential to be increased. Simms et al. (1988) displayed increased HCW of 9 and 15 kg for steers that received one or two zeranol implants during finishing when compared to control steers, respectively. For this study, no improvements were made to DP or LM area as a result of implanting during this time period. Samber et al. (1996) displayed greater final BW up to 58 kg, but similar HCW and DP for implanted steers when compared to control steers. The LM area for steers receiving 3 REV-S implants was increased when compared to the control steers. Loy et al. (1988) observed no difference in carcass measures of HCW, DP, LM area, and YG for steers that were implanted once or twice during finishing. DeHaan et al. (1990) reported improved YG by 13.5%, but no other differences

for heifers that had received two implants during finishing. Holstein steers that received different types of implants across finishing were evaluated for the effect on carcass traits (Apple et al., 1991). Steers that received an implant containing E₂, EP, or TBA in combination with these compounds previously listed had increased HCW when compared to control and TBA only implanted steers. Dressing percentage and YG were not impacted by implant treatment; however, LM area was increased for the implant treatments of EP, TBA and EP, and TBA and zeranol. Rumsey et al. (1992b) reported increased HCW by 16.1 and 19.3 kg for steers that were reimplanted and initially implanted when compared to the control steers, respectively. The delay implanted steers had similar HCW to the other implant treatments and the control steers. The authors also reported no difference in DP, YG, or LM area due to implant treatment. Roeber et al. (2000) examined the impact of implant strategy on carcass characteristics. The implant strategies for this study consisted of an initial implant on d 0 of Component T-S, Ralgro, REV-S, or SYN-S followed by a second implant for the assigned treatments of SYN-Plus or REV-S. The steers that received an implant had increased HCW and LM area, regardless of implant treatment. Scheffler et al. (2003) reported increased HCW of 36 kg and LM area by 8.8 cm² for steers that were implanted three times when compared to the controls. Reimplanting steers in this study improved the carcass yield as the once implanted steers had similar HCW and LM area to the control steers which were decreased when compared to the steers implanted three times. Dressing percentage and YG were not impacted by implantation in this study. Bryant et al. (2010) observed the effect of reimplanting during the finishing period on steer performance. Steers that received two implants during finishing had 12.6 heavier HCW, 0.3% greater DP, 3.3 cm² larger LM area, and similar YG when compared to the single implanted steers. Woerner et al. (2011) reported 11 kg heavier HCW and 3.1 cm² larger LM area for steers implanted twice

rather than only once during the finishing period. Parr et al. (2011) observed increased HCW of 6 kg, LM area by 3.8 cm², and DP by 0.35% for steers that were reimplanted instead of receiving a single implant at processing. In a second experiment, Parr et al. (2011) reported similar HCW and DP for steers that received one or two implants during the feedlot phase.

Faulkner et al. (1989) reported similar carcass characteristics except for the tendency for LM area to be larger for HT carcasses. For cows implanted with TP, no improvements were displayed for HCW, DP, or LM area; however, the weight of the *Semitendinosus* muscle was reduced due to TP implants. Heifers that received a combination implant during the feedlot phase reported increased HCW by 24.5 kg, but no other carcass traits were impacted. Moran et al. (1991) reported heavier HCW by 12 kg for heifers that received an implant containing TBA multiple times during finishing. Mader and Lechtenberg (2000) observed increased HCW of 8 kg for heifers that received two implants when compared to single implanted heifers. Other carcass measures of DP, LM area, and YG were similar between the two treatments. Sissom et al. (2007) reported similar HCW, LM area, and YG for heifers that were implanted once or reimplanted during the finishing period. Schneider et al. (2007) examined the effect of utilizing one or two implants during finishing on carcass characteristics of feedlot heifers. The heifers implanted twice resulted in an additional 6.0 kg of HCW, 5 cm² of LM area, and improved yield grade of 8% when compared of single implanted heifers.

Multiple implants during growth has the potential to drastically affect adipose measurements (Platter et al., 2003). Hopkins and Dikeman (1987) observed similar marbling score for implanted steers and bulls when compared to the control bulls. When observing 12th-rib FT, implanted bulls had 0.3 cm less fat than implanted steers but did not differ from the control bulls. Simms et al. (1988) displayed no difference in 12th-rib FT between treatments, but

observed a decreased quality grade of 8.8 or 6.1 % for steers that receive two implants during finishing when compared to steers that received zero or one implant during this period, respectively. This study displayed a greater decrease in quality for steers that were re-implanted when compared to only single or non-implanted steers. In contrast, Loy et al. (1988) observed no difference in quality grade, marbling score, or 12th-rib FT due to re-implanting steers. Similarly, DeHaan et al. (1990) reported similar FT and marbling score for heifers that received two implants during the feedlot phase. Also, Apple et al. (1991) observed similar FT and marbling score for Holstein steers receiving different types of implants multiple times across the finishing period. Rumsey et al. (1992b) reported no difference in marbling score or quality grade, but observed a 0.3 cm decrease in FT for steers that were reimplanted when compared to the initially implanted steers, while both of these treatments were similar to the control and delay implanted treatments. Samber et al. (1996) reported decreased marbling score by 57 units for steers that received three REV-S implants and were supplemented with 12.5% crude protein, while steers that received the same implant strategy had 14.5% crude protein in the diet had similar marbling scores when compared to controls. In contrast, the FT was not different due to implant treatment. The percentage of choice and prime carcasses was decreased by 31% for steers that received three REV-S implants and only 12.5% protein in the diet when compared to non-implanted steers. When supplemented only 12.5% protein, the magnitude of decrease for marbling score and percent choice and prime carcasses as a result of three REV-S implants was much greater than the steers' supplemented 14.5% protein which produced similar adipose traits as the control carcasses. Roeber et al. (2000) also examined the impact of implant strategy on beef quality characteristics. The effect of implanting was not demonstrated for FT, but marbling score was greatest for the control treatment as implant treatment caused a decrease for this measure

regardless of strategy. When considering implanting strategy, there was no clear effect of reimplanting as single implanted and reimplanted steers had similar marbling scores. The percentage of carcass grading Prime and choice was decreased for steers that received an initial implant only and reimplanted steers. Scheffler et al. (2003) reported similar FT but decreased marbling score for steers implanted once with a combination implant when compared to the control steers. Interestingly, the steers implanted two or three times reported similar marbling scores to control steers that did not receive an implant. In contrast, Platter et al. (2003) observed increased marbling scores for steers that did not receive an implant until the finishing phase of production. Also, the marbling score was increased for steers that received only two implants, when compared to those that received four or five. The percentage of carcasses that graded Choice and Prime did not differ due to treatment. Bryant et al. (2010) reported no difference in FT or marbling score when steers were subjected to single or reimplant strategies during finishing. Woerner et al. (2011) observed a tendency for steers to have decreased marbling score when implanted twice instead of once during finishing. Parr et al. (2011) reported decreased FT by 0.09 cm and marbling score by 4% for reimplanted steers when compared to once implanted steers. Also, the percentage of carcasses grading Premium Choice and Prime was decreased by 5.4% for reimplanted steers. In a second experiment, Parr et al. (2011) observed reduced quality grade for a different set of steers that were administered two implants during the finishing period.

Borger et al. (1973) reported no difference in marbling score or 12th-rib FT for heifers subjected to two zeranol implants during finishing. Faulkner et al. (1989) observed the effect of different implants strategies on quality traits for heifer carcasses. The HT implant treatment produced a decreased marbling score of 20.8% when compared to cattle that did not receive any implants. In contrast, cull cows that received the TP implant produced similar marbling score and

12th-rib FT when compared to non-implanted cows. Mader and Lechtenberg (2000) observed similar marbling score and FT for heifers that were subjected to one or two implants during the finishing period. Sissom et al. (2007) reported a tendency for decreased marbling scores for heifers subjected to a more potent initial implant and not reimplanted when compared to heifers that received a lower potency implant initially and were then reimplanted with a TBA only implant. The percent of carcasses grading Choice was decreased by 9.7% for single implanted heifers when compared to the reimplanted heifers. Similarly, heifers that receive two implants had a decreased percentage of carcasses grading Choice when compared to heifers implanted only once during finishing (Schneider et al., 2007).

Delayed Implant Strategies

The impact of time of administration of a terminal implant has been examined for its effect on feedlot performance. Rumsey et al. (1992b) examined the effect of an initial implant or delayed implant program with SYN-S on d 0 only or d 30 only on steer performance for two different trials. For the first trial, the initial and delayed implant programs had greater ADG and decreased DMI than the control treatment. The second trial displayed similar ADG and DMI for the two implant treatments, but each of these treatments had greater ADG and decreased DMI when compared to the control group. When the two trials were combined, the two implant programs performed similar for ADG and DMI, while each of them out performed the control steers for ADG and had decreased DMI. Bruns et al. (2005) examined the effect of implanting at two different time points, early or delayed, during finishing phase. When compared to the non-implanted controls, steers that received a delayed implant had increased BW by 12 kg, ADG by 5%, and G:F by 6.4% after 84 d of implantation. The early implanted cattle had similar BW, ADG, and G:F to the control and delayed implanted cattle 140 d after implantation. Regardless

of implant treatment, the implanted steers had a 10.5% increase in feed efficiency when compared to the non-implanted steers. After 57 d of implantation, the early implanted steers had similar G:F ratio to the non-implanted steers. The advantage for early implanted steers was not sustained throughout the entire study. Rather the delayed implanted steers remained more efficient for a longer amount of time than the early implanted steers. Because the delayed implanted steers would have an increased concentration of hormonal compounds later in the growth phase, this could allow them to improve growth when compared to early implanted steers that would have utilized the implant compounds earlier and when the animal is already more efficient. Munson et al. (2012) examined the effect of delaying the implant for high-risk cattle arriving at the feedlot on animal performance and health. For this study, the authors reported no advantage for health or animal performance characteristics due to delaying the implant. Gifford et al. (2015) examined the effect of early or late implanting during the finishing phase on BW of heifers. The heifers that received the early implant had increased BW of approximately 10 kg when compared to the late implanted heifer through d 84 of the study. The late implanted heifers were administered a combination implant on d 56 and had similar BW to early implanted heifers after 122 d on feed.

The effect of delaying an implant on animal performance is inconsistent; however, delaying the timing of implantation can have an effect on carcass yields and quality traits within the carcass. Bruns et al. (2005) examined the effect of delaying or initially implanting steers with a REV-S implant during the finishing period. For this study, steers that received an implant had increased HCW, DP, and LM area. The strategy of delaying the implant did not impact carcass characteristics. While lean carcass measures were not impacted by implant strategy, quality measures differed due to treatment. The steers implanted early had a decreased marbling score

when compared to the control steers but did not differ from the delayed implanted steers. In contrast, the delayed implanted steers had similar marbling score when compared to the control steers. The percentage of Premium Choice carcasses was decreased by 15.8 and 14.8% for early implanted steers when compared to control and delay implanted steers, respectively. The measure of FT did not differ due to treatment, while the percentage of intramuscular fat was decreased for the implanted steers when compared to the controls. Munson et al. (2012) reported no differences in carcass measures when high risk steers were subjected to initial or delayed implant programs, except for a tendency for delayed carcasses to have an increased value of \$0.03. The authors concluded that processing cattle a second time in order to improve the health of the animal prior to implanting does not provide any advantage to producers.

Beta-Adrenergic Agonists

Feedlot Performance and Carcass Effects

Ractopamine Hydrochloride

Several studies have displayed the effects of ractopamine hydrochloride on improving feedlot performance measures, such as ADG and G:F. Avendaño-Reyes et al. (2006) utilized crossbred steers that were subjected to 0 (**CON**) or 300 mg·head⁻¹·d⁻¹ RH for the final 33 d of the feedlot phase. Steers fed RH had a 10.6 kg increase in final BW, 24% increase in ADG, and Winterholler et al. (2007) evaluated the effect of RH administered to yearling steers which were slaughtered at three different time periods during feeding. Crossbred steers were subjected to no RH supplementation or RH supplementation at 200 mg·head⁻¹·d⁻¹ for the final 28-d of feeding. As days on feed (**DOF**) increased, animal efficiency decreased as expected; however, there was no RH × DOF interaction observed. Ractopamine did improve steer ADG and G:F by 4% over the entire feeding period, with no change in DMI. Arp et al. (2014) examined the impact of RH

at concentrations of 200 mg·head⁻¹·d⁻¹ (**RH 200**), 300 mg·head⁻¹·d⁻¹ (**RH 300**), or 400 mg·head⁻¹·d⁻¹ (**RH 400**) for the final 30 d of finishing of crossbred steers. Animal performance measures were calculated on a carcass basis. Steers supplemented RH at all levels, with the greatest response reported for the RH 300 steers, had increased carcass ADG and G:F of 15% and 13% , respectively, when compared to steers not fed RH Similar to the previous study, Bohrer et al. (2014) reported an increased ADG and G:F by 14% and 14%, respectively.

A study, utilizing heifers rather than steers, was performed by Sissom et al. (2007) to evaluate the effects of RH supplementation and DOF on feedlot performance. In agreement with Winterholler et al. (2007) there was no RH × DOF interaction. Ractopamine supplementation did improve heifer feed efficiency, but no other performance characteristics were noted. The lack of and RH × DOF interaction for the two previous studies demonstrates that RH effects are similar regardless of feeding period length. Based on the results above RH administration appears to be more prominent in steers than heifers, which was hypothesized to be due to endogenous hormone status differences between the two genders (Sissom et al. 2007). In agreement to the previous study, Quinn et al. (2008) also found a lack of feedlot performance effects for crossbred heifers. Heifers that were supplemented 200 mg·head⁻¹·d⁻¹ for the final 28 d of feeding and had received an implant during finishing had similar ADG and DMI, but tended to have improved G:F when compared to non-supplemented heifers. In a companion study, feedlot heifers that had not received an implant but were supplemented 200 mg·head⁻¹·d⁻¹ RH for 28 to 42 d during finishing had increased ADG by 14.5 and 25.3%, respectively, and tended to increased carcass G:F when compared to non-supplemented heifers (Quinn et al. 2008). Walker et al. (2006) observed the effect of RH supplemented at 0 or 200 mg·head⁻¹·d⁻¹ for 28 d in crossbred heifers. For this study, heifers had increased final BW by approximately 9 kg, ADG by 16%, and G:F by 15%. Bryant et

al. (2010) utilized crossbred heifers and fed RH for 29 d at 0 or 250 mg·head⁻¹·d⁻¹. After the 29 d feeding period, RH fed heifers had increased final BW by 10.1 kg, improved ADG by 38% and G:F by 38%. When comparing steers and heifers in the same study, Woerner et al. (2011) reported no interaction between RH treatment and sex class demonstrating a similar feedlot performance measures for both sexes in response to RH supplementation. In contrast, Walker et al. (2010) reported an interaction between RH treatment and sex class. For this study, heifers that received RH had decreased DMI when compared to control heifers, while RH and control treatment did not differ for the steer class. The authors did report that the RH fed heifers had a decreased DMI relative to the other cattle before the study and that this difference may not have been due to RH supplementation.

Abney et al. (2007) examined the effect of varying dosages (0, 100, or 200 mg·head⁻¹·d⁻¹) and durations (28, 35, or 42 days) of RH supplementation on the growth performance of crossbred steers. There was no interaction between dose and duration for all attributes measured. Linear increases occurred as dose increased for final BW, ADG, and G:F. There was a tendency for a quadratic duration response for final BW, DMI, and G:F, as these measures increased from d 28 to 35 but did not continue to increase from d 35 to 42. Average daily gain responded quadratically to RH duration, as ADG increased until d 35 of supplementation and no further increase was achieved after 42 d of supplementation. The maximal benefit for ADG and G:F, within this study, was for a RH dosage of 200 mg·head⁻¹·d⁻¹ was observed at 35 d, while the RH dosage of 100 mg·head⁻¹·d⁻¹ required a dosage of 42 d to reach the same level of performance. The authors concluded that feeding RH dosages of 100 or 200 mg·head⁻¹·d⁻¹ will improve feedlot performance, but the duration effect varied for the different dosages. Quinn et al. (2008) also tested the appropriate dosage and duration of RH supplementation in heifers. Treatments

consisted of no ractopamine (**control**); 200 mg·head⁻¹·d⁻¹ RH for 28 d (**200 × 28**); 200 mg·head⁻¹·d⁻¹ RH for 42 d (**200 × 42**); 300 mg·head⁻¹·d⁻¹ RH for 28 d (**300 × 28**); and 100 mg·head⁻¹·d⁻¹ RH for 14 d, followed by 200 mg·head⁻¹·d⁻¹ for 14 d and finishing with 300 mg·head⁻¹·d⁻¹ for 14 d (**step-up**). For all RH treatments, ADG was increased when compared to the control cattle. Along with this improvement, DMI was reduced by 6.5% for the 300 × 28 cattle when compared to the control, 200 × 28, and 200 × 42 cattle. Gain to feed ratio was unaffected by treatment for this experiment; however when contrasts were utilized, the RH groups had improved carcass G:F when compared with control. When RH was fed for 28 d at the increased concentrations (200 and 300 mg·head⁻¹·d⁻¹), the cattle responded similar to control cattle. The authors hypothesized that duration of RH supplementation could play a larger role in improving performance measurements since the increased dosages of RH performed similar at 28 d but were improved at longer duration. As duration of supplementation was increased from 28 to 42 d, carcass weight was 13.4% greater and carcasses were 4 kg heavier in the 200 mg RH cattle. Increasing the dosage from 200 × 28 to 300 × 28 only resulted in an 8.4% increase in carcass efficiency with no improvement in carcass weight. When utilizing RH, the dosage and duration can influence the response displayed by supplemented cattle.

The response to RH has been thought to be dependent on biological breed type. Gruber et al. (2007) examined the effect of RH of 420 steers from 5 different sources that represented 3 different breed types. These breed types consisted of 1) British pedigree Angus, British composites, and Angus/Hereford crossbreds; 2) Continental crossbreds: 50% Charolais/50% British-composite, 50% Limousine/50% Angus; and 3) Beefmaster steers: 37.5 to 50% Brahman/50 to 62.5% British. While the magnitude of response for feedlot performance measures was greater for Angus steers, no RH × breed type interaction was present for final BW,

ADG, DMI, or G:F. Therefore, these findings indicated that despite differences in the genetic potential of the breeds examined, the breed response to RH was the same. The effect of RH on growth performance in Holstein steers has been investigated due to their integral role in the fed-cattle market. Holstein cattle typically are larger-framed animals with smaller loin-eye areas and DP. The use of RH has been used to alleviate the undesirable characteristics associated with Holstein carcasses and improve animal efficiency. Bass et al. (2009) observed the effect of RH on implanted Holstein steers when administered $200 \text{ mg}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$ for 36 d prior to harvest. When compared to the control steers, RH improved final BW by 3% and ADG by 14%. The steers that received an implant but not RH similar final BW and ADG when compared to the steers that received RH. Vogel et al. (2009) performed a calf-fed and yearling Holstein steer study to determine the effect of RH on growth performance and carcass characteristics. For the calf fed study, RH was supplemented at 0, 200, or $300 \text{ mg}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$ for the last 28 to 38 d of finishing. The second part included a yearling Holstein steer study, in which animals received 0 or 200 mg of RH for the last 33 d of feeding. All animals in both studies received hormonal implants at least 90 d prior to harvest. Calf-fed steers fed RH at 200 or $300 \text{ mg}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$ had heavier final BW and improved ADG by 17.5 and 14.6%, respectively, when compared to the control group. When comparing the 200 and $300 \text{ mg}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$ treatments, the reduction in ADG when increasing the dosage level could be due to the decrease in feed intake of steers fed $300 \text{ mg}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$ RH. Through improvements in daily ADG and no effect on DMI, G:F ratio was improved by 15.9 and 16.6% for 200 and $300 \text{ mg}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$ RH steers, respectively, when compared to control animals. When RH was supplemented to yearling steers, RH increased DMI (4%), final BW (1%), and ADG (18.7%). Brown et al. (2014) observed the effect of RH on feedlot performance of calf-fed Holstein steers. Steers fed RH had improved ADG (4%) and

final BW (2%). There was no difference for DMI, but RH fed steers were more efficient in converting feed as they had improved G:F (12%). Woerner et al. (2011) studied the effect of RH in combination with a terminal implant for Holstein steers. There were no synergistic effects reported, but steers fed RH for the final 28 d of finishing had improved ADG (12%) and increased final BW (10 kg). The previous literature demonstrates the ability of RH to improve efficiency and carcass weights of Holstein steers in a feedlot setting.

Culled cows from beef and dairy production are another class of animals that provide a significant amount of meat to the U.S. beef industry, but the carcasses are often discounted due to poor quality, defects, and low yields (McKenna et al., 2002). Realimentation of cull cows provides a way of returning older cows to a nourished state of nutrition which results in heavier carcasses (Holmer et al., 2009b). Allen et al. (2009) studied the effects of RH and extended feeding time for market dairy cows on growth performance traits. Cows were subjected to one of three treatments: 1) control, slaughtered immediately; 2) no RH, high concentrate diet for 90 d; and 3) 90 d of high concentrate diet with $300 \text{ mg}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$ of RH for the final 32 d of feeding. As expected, feeding for 90 d before slaughter improved final BCS and final BW; however, there was no added benefit to feeding RH as no growth performance traits were affected as a result of supplementation. Holmer et al. (2009b) found similar results to the previous study after supplementing beef cull cows a high concentrate diet with or without RH at $200 \text{ mg}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$ for 35 d. Weber et al. (2013) hypothesized that the response to RH is diminished in older animals due to reduced density of β -AA and decreased sensitivity of β -AR, which may explain the lack of response seen in the previous studies. The previous authors reported no difference in feedlot performance measures of final BW ADG, DMI, or G:F for cull cows that received RH when compared to the control cows.

Improvements in feedlot performance measures provides the opportunity for carcasses from RH supplemented cattle to display improvements in carcass characteristics. Since B-AA are classified as repartitioning agents, carcasses from supplemented cattle should contain greater muscling measures at the expense of fat measures. Another study analyzing RH effects in crossbred steers which displayed improvements in ADG and G:F, also resulted in heavier carcasses and larger LM area (Gruber et al. 2007), HCW, DP, and REA, but adipose measurements are inconsistent (Boler et al. 2012, Bohrer et al. 2014, Kononoff et al. 2014). Boler et al. (2012) examined the effect of RH in crossbred steers fed 0, 200, or 300 mg·head⁻¹·d⁻¹ for 28 d prior to harvest. The steers fed RH had improved feedlot performance which translated to an increase in HCW by 4%, DP by 1%, and LM area by approximate 4 cm². Also, there was no difference between the 200 and 300 mg·head⁻¹·d⁻¹ RH fed treatments for any of the carcass traits reported. Similarly, Bohrer et al. (2014) observed an increased HCW of 2%, as a result of increased ADG during growth for steers fed 300 mg·head⁻¹·d⁻¹ of RH for 35 d prior to harvest. In contrast, these authors reported no difference in LM area or DP and tended to have an increased calculated yield grade for RH supplemented steers when compared to control steers. Kononoff et al. (2014) utilized feedlot steers that were assigned to a treatment of 0 or 300 mg·head⁻¹·d⁻¹ for 28 d. Steers fed RH had increased HCW by 1.5%, 0.7 cm² of REA, and an increased DP. Winterholler et al. (2007) observed an improvement in HCW of 2% with supplementation of 200 mg·head⁻¹·d⁻¹ RH for steers. Dressing percentage and yield grade were not affected by RH, but LM area was 1.74 cm² larger for carcasses from steers administered RH. In contrast, Gonzalez et al. (2010) did not display increases in HCW or LM area, but reported a tendency for DP to be decreased when steers were fed 200 mg·head⁻¹·d⁻¹ RH. The authors noted that the magnitude of increase in LM area was similar to other large animal number experiments that resulted in

significant differences. Therefore, the authors hypothesized the non-significant outcome for the study could be due to less animals being utilized in the experiment. Arp et al. (2014) reported increased HCW by 3.9 and 6.3 kg and DP by 0.4%, for steers fed 300 and 400 mg·head⁻¹·d⁻¹ when compared to controls, respectively. The 200 mg·head⁻¹·d⁻¹ RH treatment produced similar traits as the controls. The steers fed 400 mg·head⁻¹·d⁻¹ had increased LM area by 2.4 cm², while all other RH treatments had similar LM area when compared the controls.

Bryant et al. (2010) examined RH effects in both steers and heifers in two separate studies. The steers received levels of RH of 0, 100, or 200 mg·head⁻¹·d⁻¹ for 28 d before slaughter, while the RH heifers received 250 mg·head⁻¹·d⁻¹ for 29 d before slaughter. For steers fed 200 mg·head⁻¹·d⁻¹ RH, the authors reported increased HCW, DP, and LM area, while the 100 mg·head⁻¹·d⁻¹ RH supplemented group was similar to the control cattle. The heifers supplemented RH had increased HCW by 6.5% and increased adjusted FT by 0.12 cm, but were similar for all other carcass traits when compared to the control heifers. In contrast to the results displayed in the previous study, Sissom et al. (2007) utilized 2 larger studies investigating the RH effects on carcass characteristics. Heifers designated to a RH supplementation treatment received 200 mg·head⁻¹·d⁻¹ for the final 28 d of the feeding period. For the first experiment, animals had improved ADG and G:F which resulted in a 5 kg increase in HCW and a 2.6 cm² increase in LM area. No carcass differences were observed for the second experiment which utilized an aggressive implant strategy. The authors hypothesized that the implant status of the animal influenced the response to RH. Quinn et al. (2008) observed the effect of RH on carcass characteristics for heifers supplemented with 0 or 200 mg·head⁻¹·d⁻¹ RH for 28 d prior to harvest. This study resulted in no difference between the two treatments for HCW and LM area. In a second study, the authors observed RH fed at different dosages and durations in feedlot heifers.

Once again, the authors reported no difference between RH fed and control heifers for LM area, but increased HCW for RH fed heifers.

Endogenous estrogen levels have been hypothesized to create inconsistencies with heifer response to growth-promoting technologies. Ovariectomization and oral administration of an estrus suppressant are methods that control hormone changes and allow for improvements in response to growth-promoting technologies. Talton et al. (2014) studied the effects of ovariectomization and supplementation of RH on heifer performance. British cross heifer were stratified by weight and placed into 8 pens with 6 heifers/pen. Half of the pens were subjected to ovariectomization (**OVX**) and the other half remained intact. After OVX designation, supplementation of RH was randomly assigned to half of the pens resulting in a 2×2 factorial experimental design. The heifers that received RH supplementation were administered $0.41 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ for the final 31 d of feeding. There was no interaction between RH inclusion and sex class for any carcass characteristics traits. Ractopamine hydrochloride supplementation tended to increase HCW and LM area, as well as improve DP. There was no improvements due to OVX which is in agreement with other research findings (Vestergaard et al. 1995; Choat et al. 2006). Therefore, the additional cost of OVX did not provide producers with an incentive to utilize this method for further performance benefits in feedlot heifer production. Another method of suppressing estrus in heifers is the administration of melengestrol acetate (**MGA**), an oral active progesterone product that inhibits estrus and ovulation. Griffin et al. (2009) observed an increase in HCW of 3.3 kg for heifer supplemented MGA and RH when compared to heifers supplemented only MGA. In order to establish the role of endogenous estrogen levels on feedlot performance, a third treatment group receiving only RH could provide some evidence to any interaction that may be present for future studies.

Holstein cattle are often discounted due to undesirable carcass qualities, and would benefit from supplementation of β -AA that repartition the use of nutrients toward the deposition of lean muscle tissue. Bass et al. (2009) demonstrated the effects of RH with improvements in carcass weights of 5.7 kg and increases in LM area of 1.9 cm² in a study with calf-fed Holstein steers. Another study conducted by Vogel et al. (2009) reported no differences in yearling Holstein steers for carcass traits when subjected to RH. Allen et al. (2009) observed the effects of RH on market Holstein cows that were returned to a nourished state prior to harvest. These authors observed no differences for HCW, DP, REA, or YG measurements as a result of RH supplementation. Although inconsistencies exist for Holstein carcass characteristics, the potential to improve profitability and decrease discounted undesirable traits is still an incentive for producers to utilize these technologies.

The impact of RH on meat quality traits, such as marbling score and quality grade, have been reviewed due to the repartitioning effects of the β_1 -AA toward lean muscle accretion. For feedlot steers, Bohrer et al. (2014) reported no difference for marbling scores from cattle supplemented with 300 mg RH for 35 d. Similar to the previous study, Boler et al. (2012) and Woerner et al. (2011) observed no RH effect on marbling score. Another study conducted by Kononoff et al. (2014) administered RH at 300 mg·head⁻¹·d⁻¹ for the final 28 to 35 d of finishing and carcasses tended to have reduced marbling scores. The proportion of carcasses grading USDA Choice or better was reduced, and USDA select carcasses tended to be increased from cattle supplemented RH. Winterholler et al. (2007) reported no difference for marbling score for RH fed steers when compared to the control steers. A previous study performed by Winterholler et al. (2008) reported decreased marbling score by 4.5% as a result of RH supplementation in crossbred feedlot steers. Gonzalez et al. (2010) found that RH decreased marbling score by 3.5%

for steers fed RH. Culp et al. (2013) supplemented RH at $200 \text{ mg}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$ for 42 d prior to harvest, and reported reduced marbling scores by 9.3% and a tendency for lower quality grade. The authors hypothesized that genetic propensity for the deposition of marbling could influence the carcass's ability to capture premiums. Quinn et al. (2008) observed no difference for marbling score, but increased 12th-rib FT by 0.08 to 0.18 cm for all RH fed heifers combined when compared to control heifers. Gruber et al. (2007) analyzed the effects of RH in steers differing in biological type. It is well established that certain breeds have a greater propensity for marbling, and for this study, there was a tendency for RH steers to have decreased marbling scores regardless of breed type. Large framed dairy cattle are often at a disadvantage when compared to typical beef breeds and RH is used to improve carcass characteristics. Allen et al. (2009) observed the effects of RH in market Holstein cows, which were selected based on a high likelihood of surviving 90 d on feed. After a 24 h chill period, carcass measurements were collected and no RH effects were present for these cattle. Marbling score remained unchanged due to RH supplementation. While inconsistencies exist, the addition of RH to cattle production can negatively impact the adipose characteristics of carcasses.

Zilpaterol Hydrochloride

Approved for use in the U.S. in 2007, many studies have examined the repartitioning effects of ZH in feedlot cattle. The focus of these studies has been on performance measures, such as ADG and feed efficiency, and carcass characteristics, such as LM area and YG measurements. Plascencia et al. (1999) published one of the first studies which examined the impact of ZH on feedlot performance and carcass characteristics. The authors utilized crossbred yearling steers and supplemented ZH for 40 d with a 2 d withdrawal period. Steers supplemented ZH for 40 d had increased final BW of 20 kg and decreased DMI per kg of gain of 28%.

Avendaño-Reyes et al. (2006) reported improved ADG by 26% and G:F by 27%, but no difference for DMI for crossbred steers supplemented ZH when compared to control cattle. Similar to the previous study, Elam et al. (2009) reported increased final BW by 8 kg, ADG by 3% and G:F by 3% for crossbred beef steers supplemented ZH for 20 d compared to control steers. In contrast, Parr et al. (2011b) observed the effect of ZH in crossbred steers and saw a tendency for ADG increase, while DMI did not differ and G:F was improved for ZH fed steers. Holland et al. (2010) observed no difference for final BW or ADG, but reported decreased DMI by 4% and improved G:F by 12% steers fed ZH. Montgomery et al. (2009b) examined the effect of ZH supplemented for 0 d at 8.3 ppm concentration to crossbred steers with or without monensin and tylosin supplementation. The authors reported a tendency for an interaction for ZH \times monensin/tylosin supplementation for final BW. The steers supplemented ZH without monensin/tylosin had heavier final BW than those steers fed monensin/tylosin with ZH. There were no other ZH \times monensin/tylosin interactions reported for performance measures. The steers supplemented with ZH had increased ADG by 3.4% and G:F by 3.9%. The authors also reported a 1.2% decrease in DMI for ZH supplemented steers. Montgomery et al. (2009a) performed a study to determine the effect of ZH on performance in both feedlot steers and heifers. Final body weight was increased by 12 kg for steers and by 7 kg for heifers. Average daily gain was increased by 36% for steers and 18% for heifers, whereas G:F was improved 28% for steers and 21% for heifers. The improvement in efficiency was due in part to a 2 and 6% decrease in DMI for steers and heifers, respectively.

Vasconcelos et al. (2008) evaluated the effects of duration of ZH and DOF prior to harvest on performance and carcass characteristics in crossbred feedlot steers. Sixteen treatments consisted of ZH supplementation at 8.3 ppm for 0, 20, 30 or 40 d and DOF prior to harvest of

136, 157, 177, or 198. For this study, steers had increased ADG of up to 4.9% for all duration lengths of ZH supplementation, as well as, decreased DMI of up to 1.5% and improved G:F of up to 15.3% when compared to the non-ZH supplemented steers. Rathmann et al. (2012) explored the effect of days on the finishing diet and ZH in beef heifers on animal performance. Zilpaterol hydrochloride was supplemented at 8.3 ppm for 20 to 22 d with a 3 to 5 d withdrawal period. The effect of ZH supplementation was observed through increases in ADG by 8.7% and G:F by 11%, along with decreases in DMI by 2% when compared to the control heifers. Upon supplementation, ZH is rapidly metabolized and cleared from the body. Therefore, extending the withdrawal period past the mandatory 3 d requirement could result in a loss in performance (Rathmann et al., 2012). Robles-Estrada et al. (2009) evaluated the risk of extending the preslaughter withdrawal period on growth performance of feedlot heifers. Heifers were subjected to ZH for 0 or 30 d with a 3, 6, or 12 d withdrawal period. Dry matter intake was not affected for animals in the study. For animals subjected to ZH and a typical 3-d withdrawal period, ADG and G:F were improved by 37 and 36% when compared to control animals that did not receive ZH. Extended withdrawal periods tended to decrease carcass-adjusted ADG and G:F; however, these measurements could be confounded due to different DOF and compositional endpoints.

The addition of ZH to finishing diets can improve the amount of lean deposition in breeds such as the Holstein that lack muscularity. Beckett et al. (2009) observed the effect of ZH supplementation for 0, 20, 30, or 40 d at a constant concentration of 8.3 mg/kg during the feedlot phase for Holstein steers. When comparing the steers fed ZH for 20 d to the 0 d treatment, there was a tendency for HCW initial BW to differ, no difference in final BW, ADG, or DMI, and the G:F ratio was increased for ZH fed steers. In contrast, the steers supplemented ZH for 30 or 40 d had increased final BW by 5.4 and 13.4 kg when compared to the steers fed ZH for 0 d,

respectively. The authors reported a linear response for final BW, as the length of ZH supplementation caused an increase in final BW. Also, steers fed ZH for 30 or 40 d had an increased ADG of 5% and G:F of 8%, while DMI was decreased by 3%. There was a linear response for ADG and G:F as length of ZH supplementation increased for steers. Improving the efficiency and final BW of Holstein steers can create improvements to the carcass components of this breed type. Brown et al. (2014) examined the effect of ZH for calf-fed Holstein steer performance. For feedlot performance measures, steers that received 8.3 ppm ZH for 20 d with a 3 d withdrawal period had increased final BW by 9.2 kg, ADG by 2.5%, and G:F by 5.3% compared to the non-ZH fed steers. The previous studies provide evidence of improvements to feedlot performance for Holstein steers that were supplemented ZH during the finishing period.

Despite the inconsistent effects on growth performance measures, ZH is a potent repartitioning agent that can improve carcass yields. Plascencia et al. (1999) observed a 13 kg increase in carcass weight, a 2.2% increase in DP, and a tendency for an increase in LM area for steers fed ZH. Avendaño-Reyes et al. (2006) reported a 22 kg increase in HCW, 2 % increase in DP, and 8.5 cm² increase in LM area for steers fed ZH. Vasconcelos et al. (2008) displayed improved final carcass measurements as steers administered ZH had increased HCW by 17.2 kg and DP by 2% when compared to the controls. The LM area was 9.6 cm² larger and yield grades were reduced by 17.6% for ZH carcasses rather than control carcasses. Kellermeier et al. (2009) observed the effect for crossbred steers with or with-out supplementation of ZH at 8.38 mg/kg for the final 30 d of feeding with a 3 d withdrawal period. Steers fed ZH had increased HCW by 20.6 kg, LM area by 15.2 cm², and decreased yield grade by 27% when compared to the controls. In contrast, Elam et al. (2009) reported similar carcass measurements of HCW and DP which greater for cattle fed ZH. Montgomery et al. (2009b) examined the effect of ZH supplemented

steers with or without monensin/tylosin and observed There was a tendency for a ZH × monensin/tylosin interaction for LM area as the ZH and monensin/tylosin fed steers had decreased LM area. Also, the authors reported an increase of 13 kg in HCW, 1.2% in dressing percentage, and 8.0 cm² in LM area for ZH fed steers. The steers fed ZH had a decreased percentage of 22% for total liver abscesses when compared to the steers that did not receive ZH. Parr et al. (2011b) did not observe differences in carcass characteristics for ZH fed steers, except for an increase in HCW by 19 kg when compared to the control steers. Similarly, Rodas-González et al. (2012) reported an increase in HCW of 27 kg, but also observed an increase in LM area of 12 cm² and an improved yield grade for ZH supplemented steers. Rathmann et al. (2012) observed an increase of 38% in final BW and 3.2% in HCW. Other carcass measurements that were affected by ZH include an increase in DP by 1.5%, LM area by 5.6 cm², YG by 9.1%, a decrease in BF of 0.08 cm, KPH by 0.03%, and a tendency for a decrease in marbling score. Another study performed by Montgomery et al. (2009a) observed the effect of ZH in both steers and heifers. This study displayed a 16.4 and 12.1 kg increase in HCW, 1.5% increase in DP, and an 8.23 and 6.37 cm² larger LM area for steers and heifers, respectively. Back fat and KPH measures were unaffected for both steers and heifers in this study. Yield grade was decreased by 11.9% for steers and 9% for heifers as a result of the enhancements in lean muscle tissue and no effects on fat measurements. A greater increase in HCW was observed when compared to final BW which suggests a shift in mass from non-carcass to carcass tissues with supplementation of ZH. As duration of ZH increased, the percentage of carcasses with a large LM area increased linearly.

Other authors also found improvements in carcass cutability as ZH increased total saleable yield and improved subprimal weights (Garmyn et al. 2010; Haneklaus et al. 2011).

Shook et al. (2009) examined the effect of ZH supplementation and withdrawal periods of 3, 10, 17, and 24 d prior to slaughter on carcass characteristics. The steers subjected to a 3 d withdrawal period had the lightest carcasses when compared to the other withdrawal periods; however, the authors added that this decrease in carcass weight was potentially due to the other treatments being fed for a longer period. Due to the quick clearance of ZH from the body, drug withdrawal period could be an important factor in explaining the inconsistencies observed in animal response. The authors reported heavier carcass side by 3.3 kg and primal weights for ZH supplemented steers. For this study, most of the heavier primals were located on the hindquarter rather than the forequarter, such as the strip loin, top sirloin butt, and eye of the round. Rathmann et al. (2009) reported increased percentage of chuck, rib, strip loin, and round subprimals. Boler et al. (2009) reported increased chilled side weight for carcasses from steers fed ZH for 20, 30, or 40 d when compared to the steers fed ZH for 0 d. The primal weights for the knuckle, top and bottom round, eye of the round, strip loin, and sirloin were increased in weight for ZH carcasses when compared to the control carcasses. This increase in primal weight led to an increase in saleable meat, improving the profitability of the ZH carcasses. Hilton et al. (2010) performed 7 independent feeding trials in which ZH was fed to crossbred steers at 0 or 8.3 ppm with withdrawal periods of 3, 10, 17, or 24 d prior to harvest. For animals fed ZH, increases in CSW were observed which translated into improvements in subprimal weights from the chuck and the round. Other cuts that had increased yields were the strip loin, top butt, ball tip, tri-tip, flank steak, and peeled tender. With improvements to subprimal weights, it is vital that these increases occur to valuable cuts rather than invaluable portions of the carcass.

The effect of ZH on increasing carcass lean components is beneficial to breeds of cattle that lack in muscularity, such as the Holstein breed. Brown et al. (2014) examined the effect of

ZH supplementation on carcass characteristics of Holstein steers. For steers subjected to ZH, the HCW was increased by 15.5 kg, dressing percentage by 1.5%, and LM area by 7 cm². In contrast, Garmyn et al. (2010) observed no difference between control and ZH fed Holstein steers for HCW and yield grade measures, but reported a tendency for LM area to be increased. Martin et al. (2014) observed increased HCW by 12 kg, LM area by 7 cm², and calculated yield grade by 7% for Holstein steers supplemented ZH. Beckett et al. (2009) displayed increased HCW by 11.6 kg, and as a result, the percentage of lightweight carcasses was decreased by 5% for Holstein steers supplemented ZH for 20 d. Also, the authors reported increased dressing percentage by 1.6%, LM area by 5.1 cm², and decreased calculated yield grade by 4.9% for ZH fed steers. When the duration of ZH was increased to 30 or 40 d, steers produced heavier HCW by 13 and 17.2 kg, increased DP by 1.5 and 1.4%, increased LM area by 8.9 and 8.5 cm², and decreased YG by 10.8 and 11%, respectively.

Garmyn et al. (2010) examined the carcass cutability of ZH fed Holstein steers. For ZH fed steers, the carcasses had an increased saleable yield of 4.9 kg or 2.2%. Of the 2.2% increase, three cuts from the fore quarter (Shoulder clod, inside skirt, and back ribs) were increased in weight, while ten cuts from the hind quarter (Knuckle, top inside round, bottom round flat, eye of round, heel meat, strip loin, top sirloin butt, bottom sirloin tri-tip, peeled tenderloin, and shank meat) were increased in weight. Haneklaus et al. (2011) evaluated the differences in retail yields resulting from supplementation of ZH to Holstein steers and beef steers due to the economic advantage for the boxed-beef sector with improvements to subprimal weights. This study consisted of 3 phases in which subprimals were collected from either Holstein carcasses or beef-type carcasses. Zilpaterol fed animals had increased chuck rolls and top rounds subprimal weights which appeared to convert into retail salable advantages. Other subprimals that reported

heavier weights included the top round, outside round, and eye of the round. The heavier subprimals as a result of feeding ZH provides cutability advantages predominantly through the carcass-to-subprimal conversion. Martin et al. (2014) observed the effect of ZH supplementation for 20 d at 8.3 mg/kg for Holstein steers. The impact of ZH on carcass yield was measured through weights of the subprimal cuts of carcasses. For the ribeye roll, ZH carcasses had increased pretrim and trimmed subprimal weight. As a result, there was a greater number of steaks and an increased steak weight from ZH ribeye rolls when compared to controls.

Market cull cows account for approximately 18% of all beef slaughtered and 14.5% of beef produced in the U.S. This sector of the beef industry is an integral component, but the beef is often discounted due to poor quality traits. Lowe et al. (2012) selected cull cows that were deemed acceptable for treatment and subjected them to a concentrate diet for 42 d or concentrate diet for 19 d then ZH supplementation for 20 d. Following the feeding period, animals were slaughtered and carcass measurements were recorded. When analyzing the carcass, ZH-supplemented cows had greater DP and LM area, as well as improved YG, but no other meat quality characteristics were affected by ZH treatment. The improvement in carcass characteristics was despite the cattle lacking a response to ZH on the feedlot performance phase. Further examination of the effect of ZH on return profit displayed a \$42.34 advantage over control-fed cows as a result of improved lean muscle yields. Other authors have also noted the predominant effects of ZH on cull cow carcass composition (Neill et al., 2009; Strydom et al. 2010; Lawrence et al., 2011).

Fabrication yields are improved as a result of ZH supplementation which was displayed by Lawrence et al. (2011) through increases in yields for the rib-eye roll, top sirloin butt, top round, and peeled knuckle. Howard et al. (2014a) examined the carcass cutability of calf-fed

Holstein steers fed ZH for 21 d with a 5 d withdrawal period prior to harvest. The use of ZH increased the percent saleable yield of whole-muscle cuts by 1.95% when compared to the non-ZH fed cattle. When observing the percent change from the control, the ZH carcasses had an increased round and loin saleable yield of 1 and 0.2% while the rib and chuck were decreased by 0.2 and 0.3%. The variation in response to ZH could indicate a difference in muscle response, because the muscles in the round may have a greater sensitivity due to the high concentration of type II fibers. Supplementation of ZH can improve lean muscle yields and increase the percent saleable yield of certain cuts.

As nutrients are being deposited towards lean muscle accretion, the amount of fat that is being deposited on the animal is significantly decreased for ZH supplemented cattle. Claus et al. (2010) measured the percentage of intramuscular fat within the LL for cattle subjected to ZH supplementation for 0, 20, 30, or 40 d prior to harvest. These authors demonstrated no change to the amount of intramuscular fat within the muscle due to ZH supplementation. Another measure of adipose consists of marbling score or FT, which is measured by a USDA grader following a 24 to 48 h chill period. Zilpaterol hydrochloride administered to feedlot heifers did not affect marbling score or FT (Robles-Estrada et al., 2009). Other authors also reported no change to marbling score for cattle subjected to ZH (Neill et al., 2009; Parr et al., 2011a; Rathmann et al., 2012). Similarly, Hales et al. (2014) observed no change in marbling score for feedlot steers. In contrast, Montgomery et al. (2009b) reported that steers fed ZH had a decreased 12th-rib FT by 8.4%, KPH fat percentage by 0.1%, and marbling score by 5.7%. The ZH × monensin/tylosin interaction was significant for marbling score for this study. Removing monensin/tylosin from the diet resulted in a greater decrease in marbling score than when monensin/tylosin remained in the diet for ZH supplemented steers. Rodas-González et al. (2012) observed no difference in FT

or marbling score for steers fed ZH when compared to control steers. In contrast, Holmer et al. (2009a) displayed differences in marbling score for steers administered ZH, as loin-eyes from ZH cattle had decreased amounts of marbling. Another study conducted by Baxa et al. (2010) detected decreases in marbling score of 4.4% and FT of 1.3% for steers supplemented ZH for the last 30 d of feeding with a 3 d withdrawal period prior to harvest. Vasconcelos et al. (2008) reported decreased marbling score by 9.7%, FT by 0.2 cm, and KPH by 0.1% when compared to the controls. In contrast, Holland et al. (2010) only revealed a tendency for marbling score to be decreased and FT was unaffected for the steers subjected to ZH. Montgomery et al. (2009a) examined the effect of duration of ZH supplementation and observed a tendency for a duration \times ZH interaction and duration effect, and a ZH effect for marbling score and quality grade. As duration of ZH increased, the magnitude of the ZH effect was greater as marbling score was drastically decreased by 7.9% for the steers supplemented ZH for 40 d when compared to the 20 d period. Regardless of duration of ZH, marbling score was decreased as a result of ZH administration. Elam et al. (2009) displayed a linear trend for marbling score as ZH duration increased, the amount of marbling decreased. When compared to the 0 d supplemented group, steers supplemented ZH for 20, 30, or 40 d had decreased marbling score by 3.3, 6.5, and 7.4%, respectively. The authors also reported a tendency for a linear decrease in FT by 0.1, 0.13, and 0.14 cm for the 20, 30 and 40 d ZH supplemented steers, when compared to the controls. In a study conducted at 3 different commercial feedlots utilizing both steers and heifers, Leheska et al. (2009) reviewed the effects of ZH supplementation on meat quality and palatability traits. For this study, percentage of fat and moisture were not affected due to ZH supplementation. Lawrence et al. (2011) displayed decreases in marbling score, but ZH had no effect on FT. The ZH fed cull cows had a 6% decrease in marbling score when compared to non-ZH fed cull cows.

Zilpaterol hydrochloride is a potent β -AA that can decrease marbling up to 23 units and FT up to 0.11 cm (Lean et al., 2014).

The potency of ZH is greater when compared to RH; therefore, the animal efficiency and carcass traits are expected to be more prominent. A recent study conducted by Brown et al. (2014) compared the two β -AA effects in Holstein steers. No difference between treatment groups was observed for initial BW, BW at 28 d, or DMI. After 28 supplementation of both β -AA, the steers had increased BW and improved overall ADG and G:F. For carcass traits, the steers supplemented with RH and ZH had increased HCW by 8.2 and 15.5 kg and LM area by 2.3 and 7.1 cm², while only ZH carcasses had increased DP by 1.5%. There was a 6.1 and 7.3% increase for percentage of YG 1 and 2 carcasses, respectively, for steers fed when compared to control. The RH carcasses had similar percentages of YG 1 and 2 as the controls, but had decreased percentages of these carcasses types when compared to the ZH carcasses. The authors reached the conclusion that supplementation of ZH offers advantages to both the producer and packer through improvements on both the feedlot and carcass components. In contrast, Scramlin et al. (2010) compared the effects of both RH and ZH on performance and carcass characteristics in finishing steers. During the feedlot phase, steers supplemented RH or ZH had increase final BW of 7.5 and 3 kg, respectively. Also, these supplemented steers reported increased ADG and G:F when compared to the control steers. Van Donkersgoed et al. (2011) compared the effects of RH and ZH for feedlot heifer performance and carcass characteristics. The heifers supplemented RH or ZH did not differ for any performance measure, except for a 3.1% decrease in DMI for ZH heifers. For carcass characteristics, the ZH heifers had 11.6 kg greater HCW, 1.6% increase in DP, 5.7% increase in YG 1 carcasses, and 0.9% decrease in Prime carcasses when compared to RH supplemented heifers. Martin et al. (2014) examined the effect of no β -AA, RH, or ZH

supplementation in Holstein steers. The authors reported increased HCW by 6.6 and 12.9 kg and LM area by 3.1 and 7 cm² for RH and ZH fed steers when compared to the controls, respectively. When compared to one another, the ZH fed steers had a greater HCW by 6.3 kg and LM area by 3.9 cm², while yield grade remained similar to the RH steers' carcasses. Further examination of the primal weights resulted in an increase in ribeye roll weight by 0.2 kg for the ZH carcasses, while the RH was similar to both the control and ZH carcasses; however, the strip loin weight was increased for both RH and ZH carcasses by 0.12 and 0.37 kg, respectively, while the ZH strip loin was 0.25 kg heavier than the RH loin.

Combined Use of Beta-Adrenergic Agonists and Implants

In order to maximize the effect of growth-promoting technologies, β -AA are often used in combination with several implant strategies. It has been thought that the effects of one could impact the effect of the other through similar growth pathways leading to synergistic effects. The abundance of research displays different pathways of growth for each growth-promoting technology, which results in additive effects of combining the technologies during the growth phase of cattle. Sissom et al. (2007) observed the effect of RH in combination with several implant strategies on feedlot performance of feedlot heifers. The experiment was set up as a 2 \times 2 factorial with the main effects consisting of implant treatment and RH. Although not specifically stated in the article, the authors did not report an implant \times RH interaction for this study, demonstrating that the two GP work through separate growth mechanisms. Winterholler et al. (2008) utilized the same experimental design but examined the effects of a convention (monensin, tylosin, and implant) vs natural (no monensin or tylosin with or without RH) system on steer performance. There was a tendency for adjusted overall ADG to display a management system \times RH interaction, while adjusted overall G:F displayed this interaction. In a second

reported study, there was a system \times RH interaction displayed for overall ADG and overall G:F. The steers that received an implant and RH had 24 and 19% greater ADG and G:F, respectively, when compared to the implanted steers not supplemented RH. When examining the natural system, steers that received RH had a 25% decrease in ADG and G:F when compared to the non-RH supplemented steers. The removal of monensin, tylosin, and an anabolic implant from the natural system caused negative responses for the RH fed steers, when compared to their control counterparts. Bass et al. (2009) observed the effect of RH in combination with various implant regimens on carcass characteristics of Holstein steers. When the interaction was examined, the steers did not report an implant \times RH interaction for HCW during this study. No other measures were examined for the implant \times RH interaction. Bryant et al. (2010) conducted two separate experiments to determine the effects of RH and steroidal implants on feedlot performance and carcass traits for steers and heifers. The first experiment utilized steers that were subjected to 3 different implant treatments (no implant, initial implant, or re-implant) and 3 different levels of RH (0, 100, or 200 mg·head⁻¹·d⁻¹). For feedlot performance measures, there was no implant \times RH interaction present for the steers, but when examining carcass traits there was a tendency for an implant \times RH interaction for KPH percentage and skeletal maturity. The second experiment within this study utilized heifers that received no implant, an implant containing TBA only or in combination with estradiol, and 0 or 250 mg·head⁻¹·d⁻¹ of RH. Similar to the steers, no interactions were present for feedlot performance measures, as well as the carcass characteristics. Woerner et al. (2011) evaluated the effects of growth-promoting technologies during each stage of finishing in crossbred calves. Implants were administered on d 0 for treatment 2, 3, and 4 and on d 63 for treatments 1, 3, and 4. Ractopamine hydrochloride was supplemented for the final 28 d to animals in treatment 4 only. Growth-promoting treatment for this study elicited similar

effects, demonstrating that there are no synergistic effects when implantation and β -AA are used together. For group 4, animals had improved ADG for the final 28 d, LM area, and yield grades after being subjected to 2 implants and RH.

Kellermeier et al. (2009) examined the effect of ZH and a terminal implant containing TBA and estradiol on carcass yield of crossbred steers. For all carcass measures, there was no implant \times ZH interaction for HCW, yield grade, LM area, and adipose measures. Steers fed ZH had increased HCW by 20.6 kg when finished without an implant and 10.4 kg when finished with an implant. Also, steers had increased LM area by 15.2 and 11.1 cm² and decreased yield grade 27 and 18% when fed ZH with or without an implant, respectively. When examining subprimal yields, the authors reported an implant \times RH interaction for the yield of bottom sirloin butt, ball tip, tri-tip, and the flank steak. The authors concluded that ZH produced an additive effect for the terminal implant for subprimal yields. Parr et al. (2011) evaluate the effects of utilizing an implant containing TBA and E₂ and feeding ZH on performance and carcass characteristics of beef steers. There was no implant \times ZH interaction for feedlot performance or carcass characteristics for this study. Another recent study performed by Parr et al. (2014) analyzed the effect of TBA and E₂ implants with or without ZH supplementation on serum hormones and metabolite concentration. There was no interaction between implant and ZH for any of the variable measured, once again supporting the idea that these growth-promoting technologies work through different pathways. These authors found similar results to a previous study performed by Baxa et al. (2010). The treatments consisted of a control group (no implant or ZH), only ZH supplementation, only implant administered, and an implant and ZH administered group. Zilpaterol hydrochloride supplementation increased ADG by 5.7%, G:F by 6.4%, HCW by 21.5 kg, DP by 2.5%, and LM area by 11.6 cm², but the ZH and implant group

had the greatest increase for ADG and G:F by 12.6 and 11.1% when compared to the non-implanted and non-ZH supplemented steers. The authors concluded that the increase appeared to be additive when compared to the other separate treatments. Combining the use of implants and β -AA allows producers to capitalize on technologies that improve growth efficiency measurements for an industry striving to cut back on production costs to improve profitability.

Growth-Promoting Technologies Impact on Meat Tenderness

Biological Contributors to Meat Tenderness

Postmortem beef tenderness is determined by two major factors: myofibrillar tenderness and collagen characteristics. The myofibrillar component tenderness is determined by the skeletal muscle characteristics and the amount of postmortem degradation that occurs as a result of postmortem aging (Koohmaraie and Geesink, 2006). The collagen component is often considered the background toughness, which is the minimal toughness that can be achieved following postmortem aging (Blanco et al., 2013). Other postmortem factors that can influence beef tenderness are length of aging period (Tatum et al., 1999), quality grade or amount of marbling (Wheeler et al., 1994), and sample location within a muscle (Alsmeyer et al., 1965).

The first determinate of tenderness is the myofibrillar fraction which consists of skeletal muscle characteristics. Skeletal muscle is a heterogeneous tissue that consists of several structures that enable movement and provide support for the animal. The whole muscle consists of muscle bundles which are made up of individual muscle fibers, the cellular unit of skeletal muscle. Each muscle fiber consists of repeating units known as sarcomeres, which provide the structures responsible for muscle contraction (Forrest et al., 1985). Because the number of muscle fibers remains the same after birth, growth of skeletal muscle must occur through hypertrophy rather than hyperplasia. An increase in hypertrophy of muscle fibers causes an

increase in cross-sectional area (**CSA**). Adult bovine skeletal muscle consists of three fiber types that differ based on their contractile and metabolic properties (Chikuni et al., 2004). The major protein responsible for the fiber type profile is the myosin heavy chain isoforms (**MHC**; Schiaffino and Reggiani, 1996) which consists of: MHC type I, a slow-twitch, oxidative; MHC type IIA, a fast-twitch, oxidative and glycolytic; and MHC type IIX, fast-twitch, glycolytic. Typically, the order of fiber size from largest to smallest is IIX>IIA>I, because type I fibers must remain smaller to maintain the oxidative metabolism (Maltin et al., 2003). The muscle fiber type distribution will vary from muscle to muscle (Kirchofer et al., 2002). As muscle converts to meat postmortem, the fiber characteristics within a muscle can affect meat tenderness and quality traits (Chriki et al., 2012). An increase in muscle fiber CSA can impact tenderness measurements (Crouse et al., 1991; Dransfield et al., 2003; Chriki et al., 2012). Seideman et al. (1987) reported a correlation between fiber size and shear force. Crouse et al. (1991) observed a correlation between shear force and fiber size at early periods of postmortem aging, but this correlation was not present in for samples aged to 6 or 14 d. The authors concluded that fiber size could be a contributor to tenderness prior to postmortem proteolysis, but the process of proteolysis during storage helped eliminate the effects of fiber size shortly after slaughter. The use of GP can have potential influences on the fiber characteristics within skeletal muscle and can affect the final tenderness of beef products. The myofibrillar component has been reviewed extensively in order to determine its impact on meat tenderness, but the collagen fraction has not been analyzed as in depth and provides an area for further research.

Collagen is one of the major proteins that makes up 1 to 10% of dry matter in skeletal muscle (Bendall, 1967) and provides support for the muscle fibers and helps transmit force to the skeleton producing movement (Bailey, 1985; Purslow, 2005). The molecular structure of

collagen is described as a triple alpha-helix which consists of three single chain helix strands with repeating units of glycine-proline-hydroxyproline-glycine-one of the other amino acids. Due to the glycine falling at every third residue, the triple alpha-helix can be packed very close together forming a tropocollagen molecule. This molecule has a molecular weight of 300,000 and is 280 nm in length (Weston et al., 2002). The precursor for collagen is the fibroblast which synthesis precursors for the extracellular components of collagen. Collagen consists of mostly extracellular components consisting of glycoproteins and end products of connective tissue metabolism. The most abundant amino acid that makes up collagen is glycine accounting for approximate one-third of the composition. Hydroxyproline and proline contribute another one-third and are present throughout the tissue at a relatively constant rate. Due to the constant composition, hydroxyproline is commonly used as an indicator of connective tissue in meat products (Lawrie and Ledward, 2006). At least 12 different types of collagen have been identified but only type I, III, IV, V, VI, XII, and XIV are present in muscle tissue. The predominate types of collagen are type I and III. The collagen network consists of three layers: the endomysium, perimysium, and epimysium (Purslow, 2014). The epimysium surrounds the whole muscle and is often removed during fabrication. Next, the perimysium is present between muscle bundles and is the major contributor of meat toughness. The endomysium coats the individual muscle fibers and is typically associated with 20 to 40 muscle fibers which are grouped together to form primary muscle bundles. Primary muscle bundles are grouped together to form secondary bundles and the perimysium surrounds both of these to muscle bundles. Each layer is composed of mostly type I and III collagen, which are fiber forming types of collagen, while trace amounts of type IV are found in the basement membrane of the endomysium layer attaching the connective tissue to the sarcolemma of the associated muscle fiber (Purslow, 2005).

These layers are present throughout the entire muscle as a continuous network that must be remodeled during growth to allow for muscle hypertrophy (Lawrie and Ledward, 2006; Purslow, 2014). To achieve the strength necessary to complete the task of supporting skeletal muscle, the collagen molecules are arranged in a quarter-staggered arrangement and are bonded together with crosslinks to prevent sliding under strain (Weston et al., 2002). As an animal ages, the immature, divalent crosslinks present in collagen transition into mature, trivalent crosslinks which are associated with decreased heat soluble collagen (Bailey and Light, 1989; McCormick, 2009). The formation of additional crosslinks is achieved by reactions between collagen and glucose or aldehydes (Purslow, 2014). Two types of mature crosslinks that are formed through enzymatic crosslinking are hydroxylysylpyridinium and lysylpyridinium. Concentrations of mature crosslinks can be impacted by several factors including muscle type and growth rate. The measure of collagen turnover and synthesis is proportionate to the amount that is soluble upon heating (Rompala and Jones, 1984). Upon heating to 65°C, the collagen undergoes shrinkage, hydrolyzes, and then becomes gelatin. The heating process allows collagen to become more tender as it becomes more soluble and has a greater WHC. The amount of mature crosslinks will determine the extent of shrinkage and the amount of tension formed during this process (Forrest et al., 1975; Lawrie and Ledward, 2006). A more mature collagen sample will produce a greater amount of tension during collagen shrinkage, which results in a tougher meat product (Bailey, 1985). Field et al. (1996) revealed a correlation between the amount of crosslinking and WBSF values in steaks from heifers. Similar to the previous study, Gonzalez et al. (2014) observed increased amounts of the enzymes involved with enzymatic crosslinking for animals containing half Brahman genetics, which also correlated with WBSF. Fishell et al. (1985) observed the effect of growth rate on total and soluble collagen of the *Semimembranosus* from steers differing

in their rate of growth. There was no effect of growth rate on total collagen, but percent soluble collagen was decreased for slower growing steers when compared to faster growing steers. Archile-Contreras et al. (2010) examined the correlation between of growth rate and the solubility of the collagen fraction of LM steaks from steers. In most cases, there was a positive correlation between ADG and percentage of heat soluble collagen in LM steaks. Different diets created different results, and the authors concluded that the results from this study did not support the theory that increased growth rate improved tenderness through increased collagen solubility. As growth rate is increased, the turnover rate of collagen is increased resulting in more heat-labile crosslinks and increased solubility (Fishell et al., 1985). Since GP improve the growth rate of cattle, the turnover rate of collagen could be influenced resulting in a more soluble collagen fraction. While collagen is the most abundant protein in the body, it only accounts for approximately 2% of muscle tissue; however, meat cuts that contain a greater amount of collagen will typically produce tougher products (Bailey, 1985). Further exploration of the relationship connective tissue characteristics and meat tenderness should be done to improve the understanding of connective tissue properties and the background toughness of beef products.

In meat tissue postmortem, the proteolytic system actively breaks down skeletal muscle structures and improves meat tenderness. The system primarily responsible for degradation of the myofibrillar structures consists of the calpains (Koochmaraie and Geesink, 2006). Calpains are an intracellular Ca^{2+} -dependent cysteine neutral proteinase that are endogenous to skeletal muscle tissue. The two types of calpains that are responsible for meat tenderization include μ -calpain and m-calpain. The calpain molecules are composed of two subunits weighing 80 and 28 kDa. In the presence of calcium, the calpain molecules undergo autolysis and produce smaller molecular weight fragments for the large subunit of 78 followed by 76 kDa (Inomata et al.,

1988). Some of the proteins degraded during postmortem aging include: desmin, dystrophin, nebulin, and titin, but this system does not degrade the actin and myosin proteins. As a result of degradation, the myofibrillar structure is weakened and tenderness is improved. A natural inhibitor of calpains is calpastatin, which binds to calcium and impedes calpain degradation. Alterations to the concentration of these proteases during growth can create differences in degradation of postmortem muscle tissue. The calpain system is primarily responsible for degrading the myofibrillar structures of meat, but other proteases that break down the connective tissue fraction of meat should be explored for their effects on tenderization of meat products.

One of the major proteases that assists with breakdown and remodeling of connective tissue are matrix metalloproteinases (**MMPs**; Purslow, 2014). Ractopamine hydrochloride can cause an increase in MMP expression within myoblasts (Cha and Purslow, 2012), which provides evidence of the role that RH on collagen turnover due to muscle hypertrophy. Matrix metalloproteinases breakdown the layers of connective tissue, and cause mechanical weakening of the structures during postmortem degradation. The relationship between postmortem degradation of collagen and cooked meat tenderness is difficult to make because heating causes shrinkage of the collagen network and physical changes to the myofibrillar components (Purslow, 2014). To date, little research has focused on the collagen component of meat tenderness; however, new research has demonstrated the influence of postmortem aging on tenderization of the connective tissue network. Influencing the amount of collagen turnover and synthesis by impacting growth can change the maturity of the collagen matrix creating the opportunity to improve the background toughness of meat products.

As studies observe the effect of growth-promoting technologies on tenderness qualities, researchers must account for variations that occur due to sampling location within a muscle.

Previous studies have reported a tenderness gradient within the *Longissimus lumborum* muscle in the anterior to posterior direction, as well as medial to lateral (Ginger and Weir, 1958; Kinsman, 1961; Alsmeyer et al., 1965). In order to define the areas within the *Longissimus* muscle that are less tender, Alsmeyer et al. (1965) collected beef rib roasts and performed Warner-Bratzler shear force and slice shear force (SSF) at multiple locations. For WBSF analysis, one core was removed from the dorsal end (closest to the spinous process, one core from the medial location, and one core from the lateral location of the rib roast following a cook period when steaks were heated to 60°C. The remaining portions were chilled overnight and uniform slices were cut to 0.22-in thick. The slices were then positioned in the STE instrument and were penetrated parallel to the fiber axis. For this study, the dorsal end was the most tender when compared to the medial and lateral positions when analyzed using the STE instrument. When analyzing WBSF values, there was no difference reported between the 3 areas. The authors concluded that emphasis on selection and control of sampling locations should be carefully determined when tenderness is a considered trait. Kerth et al. (2002) analyzed the shear force gradient of the LM through WBSF determination of steaks from top loin subprimals and aged to 7 or 14 d. Steaks were thawed for 24 h prior to cooking then cooked to reach an internal temperature of 71°C. Following a 24-h chill period, steaks were cored at 10 to 15 different locations parallel to the muscle fiber orientation, depending on total LM area and connective tissue variation. The location of the cores produced different WBSF values in a lateral to medial and dorsal to ventral direction. The furthestmost lateral region of the LM steak consistently produced the greatest WBSF values than the other regions. Noted by the authors was the practice of maximizing core locations which is not recommended based on Wheeler et al. (1996) findings that demonstrated very little improvement in repeatability when more than 6 cores were analyzed from individual steaks.

Rather than maximize the amount of cores, the authors concluded the importance of consistent collection of core samples to characterize the mean shear value more precisely. Derington et al. (2011) observed the effect of location of strip loin steaks and cores within on WBSF measures. Within the loin, the authors reported that the first five most anterior steaks were similar in WBSF, but the further you move back within the loin the steaks became tougher. Due to the gradient within the loin, it is imperative to use common locations in order to reduce variation. The previous studies demonstrate how sampling location can impact tenderness measurements. In order to decrease inconsistencies, coring methods should remain consistent across studies to decrease in variation that may occur between samples.

Effect of Growth-Promoting Technologies on Meat Tenderness

Implants

The use of implants during growth improves efficiency and yields, but the effect of these technologies on meat tenderness needs to be addressed as producers strive to yield products that are acceptable to consumers. The use of anabolic implants causes muscle hypertrophy through the addition of satellite cells to existing muscle fibers. This increase in muscle fiber size can have negative impacts on meat tenderness. Borger et al. (1973) observed similar tenderness measures for steaks from steers that were implanted or not implanted during finishing. Also, Boucque et al. (1988) reported no difference in WBSF for bulls that received a cocktail of androgens and estrogens when compared to the controls. Crouse et al. (1987) observed no difference for tenderness sensory panel scores for heifers subjected to E₂, TBA, or TBA and E₂ implants. Faulkner et al. (1989) displayed no change in WBSF was observed within the *Longissimus* muscle for this study. Other authors observed no change in shear force for *Longissimus* muscle steaks from cattle subjected to implants during growth (Apple et al., 1991; Vestergaard et al.,

1995; Holzer et al., 1999; Schoonmaker et al., 2001). Hunt et al. (1991) observed the effect of TBA or combination implants on tenderness of LM steaks from steers. When administered an implant containing only TBA or a combination of TBA and E₂, steers had similar WBSF as the control steers for this study. Perry et al. (1991) reported no difference in taste panel tenderness rankings for steaks from Holstein and beef steers that had been implanted with TBA and E₂. Gerken et al. (1995) utilized cloned steers to determine the effect of implant type on palatability of strip loin and top sirloin steaks. For strip loin and top sirloin steaks, all treatment groups reported similar WBSF. In contrast to the previous studies, the use of multiple implants can reduce tenderness. Samber et al. (1996) examined the effect of multiple implants during finishing on WBSF of LM steaks. When compared to the control steaks, the steers that received two or three successive REV-S implants had increased WBSF of 0.43 and 0.31 kg after 14 d of aging, respectively. Foutz et al. (1997) observed an increase in WBSF by 0.22 kg for steers that received an implant containing TBA and E₂ compared to non-implanted controls. When steers were reimplanted, the steaks produced had 0.41 and 0.29 kg greater WBSF when compared to the non-implanted and once implanted treatments. Similar to the previous study, Scheffler et al. (2003) reported an increase in WBSF for steaks by 0.5 and 0.4 kg from steers that received three implants during growth when compared to steers that were subjected to zero or one implant, respectively. Roeber et al. (2000) observed the effect of implant strategy on WBSF of steaks from feedlot steers. When steers received an initial REV-S implant, the WBSF was increased by 0.54 kg when compared to the controls. All other implant strategies including reimplanting and delay implants, had similar WBSF to control and the REV-S initial implant treatments. Platter et al. (2003) reported an increase in WBSF for steers subjected to multiple implants during growth when compared to steers that did not receive an implant. The steers that received one implant

had an increased WBSF 0.43 kg when compared to controls and did not differ from the steers that received four or five implants during growth. Also, the steers that received three implants had a greater WBSF of 0.3 and 0.73 kg when compared to double implanted steers and nonimplanted steers, respectively. The percentage of steaks that had a WBSF less than 4.5 kg after 14 and 21 d of aging was decreased for steers that had received three, four, or five implants when compared to control steers. Kerth et al. (2003) reported increased WBSF by 0.31 kg for steaks from non-implanted heifers when compared to steaks from heifers implanted twice with REV-H. The other implant strategies in this study did not differ from one another for WBSF of LM steaks. Barham et al. (2003) demonstrated the role of postmortem aging in improving tenderness as WBSF values did not differ for steaks after 19 d aging from cattle receiving no implant or at least 2 implants. Schneider et al. (2007) reported that an aging period of at least 14 d is required to negate the effects the implants and provide consumers with an acceptable product. The threshold for consumer satisfaction should be considered when making conclusions to changes in shear force values. Smith et al. (2007) reported increased WBSF values for implanted cattle; however, steak shear force averages were below 3.0 kg on d 1 of aging which is a level that has been determined to have 100% satisfaction for consumers.

Boles et al. (2009) hypothesized that variations between breed, sex, and implant strategy contributes greatly to the inconsistencies displayed in meat tenderness of beef steaks. For the current study, shear force values were impacted by breed type and use of implant, and tended to be influenced by sex of the animal. There were no interactions present for breed \times implant or sex \times implant, indicating that the implant produced similar results regardless of breed or sex of the animal. When examining the implant effect, steers that received an implant produced steaks that had an increase of 0.6 kg in WBSF when compared to the non-implanted steers. Schneider et al.

(2007) analyzed several contrasts of implant strategy and the effect of extended aging on WBSF of LM steaks. When comparing no implants to one implant strategies, the WBSF was similar for across the aging period. The contrast of single implant to reimplant strategies resulted in a significant effect as reimplanted heifers had greater WBSF at d 3, 7, 14, 21, and 28 d of aging. The use of both TBA and E₂ was compared to the use of TBA only implants. Following 3, 7, 14, and 21 d of aging, the heifers implanted with a combination implant produced steaks with a greater WBSF than those implanted with only TBA. After 28 d of aging, the WBSF for these two types of implants strategies did not differ. Kellermeier et al. (2009) examined the effect of REV-S implants on WBSF measures across 21 d postmortem aging. On d 7 of aging, implanted steers had an increase in WBSF of 0.93 kg. This value was reduced to 0.41 kg following 14 d aging, but after 21 d the implant steaks had a WBSF that was 0.64 kg greater than control steaks. When observing WBSF values, the implant steaks reached the tenderness threshold by 14 d of aging while the control steaks reported acceptable tenderness values following 7 d of aging. Garmyn et al. (2011) examined the effect of REV-S or REV-XS implants on tenderness measures of LM steaks from beef steers. After 7 d of aging, the implanted steers tended to have steaks with increased WBSF when compared to the control steaks. Following 14 and 21 d of aging, the implanted steers produced steaks with increased WBSF by 0.23 and 0.23 kg for the REV-S, and 0.4 and 0.4 kg for the REV-XS treatments, respectively, when compared to the non-implanted controls. After 28 d of aging the REV-S implanted steers produced steaks that were similar to both the controls and the REV-XS implanted steers; yet, the REV-XS steers produced steaks that had increased WBSF by 0.43 kg when compared to the controls. Extending the aging period to 35 d allowed the control and REV-XS treatments to become similar to each other, while the REV-S steers produced steaks with 0.27 kg greater WBSF. Regardless of the differences due to

the treatments, all the steaks means for this study were under the consumer tenderness threshold of 4.3 kg. Woerner et al. (2011) observed the effect of reimplanting with a terminal implant on WBSF of LM steaks. The steaks from reimplanted cattle had similar WBSF when compared to the single implanted cattle. Girard et al. (2012) examined the effect of the myofibrillar and connective tissue fractions and the use of implants on WBSF of *Semitendinosus* steaks. When implants were administered, the myofibrillar fraction remained unchanged but the connective tissue fraction resulted in an increased WBSF by 1.04 kg. The shear force of the connective tissue fraction was also negatively correlated with percent soluble collagen.

When reviewing changes to tenderness values, the factors that influence beef tenderness should be considered. Calkins et al. (1986) reported no change to total and soluble collagen with the use of zeranol implants during growth. Gerken et al. (1995) observed a similar amount of total collagen and percent soluble collagen for LM steaks from steers subjected to zero or one implant containing TBA, E₂, or TBA and E₂. Kellermeier et al. (2009) measured the amount of total collagen in steaks from implant and nonimplanted steers. In contrast to the previous study, steers that received a REV-S implant during finishing produced steaks that had 34% less total collagen than the control steaks. Gerken et al. (1995) observed no effects on μ - and m- calpain activity, but found an increase in calpastatin, an inhibitor of the calpain system, activity for steers implanted with products containing a combination of E₂ and progesterone or TBA. The effects on calpastatin did not impact the tenderness measurements for the current study. Morgan et al. (1993) examined the effect of hormone status on the calpain/calpastatin system by utilizing bulls and castrated steers. For this study, the authors reported an increase in the level of calpastatin for bulls when compared to steers. In the live animal, the calpain proteolytic system is a good indicator of myofibrillar protein turnover and in meat postmortem, calpain activity can influence

the fractional degradation rate. The authors of the current study concluded that an increase in calpastatin activity would cause a decrease in calpain-mediated degradation and result in a decreased fractional degradation rate. The impact of implants on postmortem degradation rates can cause decreases in tenderness as displayed through increases in WBSF; however, the extent of the change can be dependent on the implant strategy utilized by producers, as well as characteristics of the population of animals. Also, the fiber size can impact the WBSF values as the GP can increase fiber size resulting in increased muscle yields. Kellermeier et al. (2009) reported increased fiber diameter for steers that received a REV-S implant during finishing when compared to the control counterparts. Another measure that can influence WBSF and can be impacted by GP is water holding capacity (**WHC**). Borger et al. (1973) reported an increased cook loss percentage by 2.3% for steaks from implanted steers compared to controls. Boucque et al. (1988) observed no difference due to implanting on WHC of LM steaks. Similarly, Kellermeier et al. (2009) reported similar cook loss percentage for steaks from implanted and control steers. The percent strip loin purge loss was increased for REV-S implanted steers when compared to the controls. Faulkner et al. (1989) reported similar cook loss percentage for steaks from heifers that received a TP implant when compared to heifers that were not implanted. Foutz et al. (1997) observed no difference in the percent cook loss for LM steaks from steers implanted or not implanted during the finishing phase. Scheffler et al. (2003) displayed no difference in percentage of cook loss for steaks from steers subjected to zero or up to three implants during finishing. Kerth et al. (2003) reported similar cook loss percentage for steaks from heifers that were subjected to up to two different implants during finishing and nonimplanted heifers. Garmyn et al. (2011) observed no difference due to implant treatment for percent cook loss

following 7, 21, 28, or 35 d of aging, but the steaks from implanted steers had an 1.2% increase in cook loss when aged 14 d.

Beta-Adrenergic Agonists

Ractopamine Hydrochloride

Many studies have analyzed tenderness of several cooked meat products through a measurement of Warner-Bratzler shear force. Avendaño-Reyes et al. (2006) observed the effect of RH supplemented for 33 d to feedlot steers on WBSF of LM steaks. When fed RH, steers produced steaks that were 0.44 kg tougher than control steaks. Martin et al. (2014) observed the effect of RH on WBSF and SSF of LM steaks aged 16 or 23 d postmortem. The RH steaks differed from the control fed steaks after 16 or 23 d of aging. After 16 d of aging, the RH steaks had a 0.41 kg increase in WBSF. Following 23 d of aging, the RH steaks remained increased but reported a 0.42 kg increase in WBSF when compared to control steaks. Bohrer et al. (2014) collected strip loins from Angus-cross steers that were subjected to RH. Loins were allowed to age for 14, 21, and 28 d postmortem then were subjected to cooking procedures. Ractopamine steaks aged 14 d and 21 d tended to have 0.43 kg and 0.25 kg greater shear force values, respectively. Shear force values were not different after 28 d aging for steaks from this study. Regardless of the increased shear force values, Martin et al. (2014) and Bohrer et al. (2014) reported averages that were below the consumer threshold for acceptable tenderness indicating that an increase in shear force for RH steaks may not be detected as different by consumers. Another study examined the effect of RH in Simmental-Angus crossbred steers on WBSF for strip loins aged 4, 7, 14, 21, or 28 d (Boler et al., 2012). On d 4, steaks from the cattle administered RH had 0.41 kg greater shear force when compared to control cattle. Following a 7 d aging period, shear force values were similar for all treatments. In contrast, Strydom et al.

(2009) examined the effects of RH on LM steak tenderness of feedlot steers. Steaks were aged to 2, 7, and 14 days postmortem and WBSF was collected following the appropriate aging period. For steers fed RH, the LM steaks had similar WBSF on d 2, 7, and 14 as the non RH fed steers. Bass et al. (2009) reported similar WBSF for Holstein steers subjected to RH for 36 d prior to harvest. Quinn et al. (2008) observed the effect of RH on LM steak tenderness of feedlot heifers. The heifers assigned to the RH treatment were fed RH at a concentration of $200 \text{ mg}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$ for 28 d. The LM steaks from control and RH fed heifers produced similar WBSF after aging to 14 d. Strydom and Smith (2010) reported similar WBSF for cull cows that were subjected to RH for 0, 20, 30, or 40 d. Allen et al. (2009) examined the effect of RH supplementation to market dairy cows on the WBSF of LM steaks. The RH fed cows produced steaks with similar WBSF as the non RH fed cows. Scramlin et al. (2010) observed the effect of RH on strip loin steak WBSF aged to 3, 7, 14, or 21 d postmortem. The RH steaks aged to 3 and 7 d had increased WBSF of 0.70 and 0.48 kg when compared to control steaks. When aged to 14 and 21 d, the RH steaks produced similar WBSF as the control steaks. Howard et al. (2014b) measured the SSF of top loin steaks from Holstein steers fed 300 or 400 $\text{mg}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$ RH, segregated by quality grade, and aged for 14 or 21 d. After 14 d of aging, the RH steaks that graded low Choice and high Choice had similar SSF when compared to the three quality grades of the control steaks. The Select steaks from the RH fed steers had increased SSF by 2.9, 3.3, and 4 kg when compared to the control Select, low and high Choice steaks, respectively. Following 21 d of aging, both the RH and control steaks had similar SSF values for all quality grades. Woerner et al. (2011) observed the effect of RH supplementation on WBSF of LM steaks. The RH fed cattle produced steaks that were 0.23 kg tougher when compared to the control fed cattle. Gruber et al. (2008) observed the effects of RH supplementation and postmortem aging on shear force of the

Longissimus muscle for cattle from 3 different biological types. Weanling steer calves were selected to represent British, Continental, and Brahman cattle types equally and were or were not supplemented 200 mg·head⁻¹·d⁻¹ RH for the final 28 d before slaughter. Strip loins were collected and measurements of WBSF and SSF were obtained following cooking procedures. An interaction between RH supplementation and biological type was present for WBSF. The magnitude of increase for WBSF was greatest for Brahman cattle with a 0.57 kg increase due to RH supplementation. Continental and British cattle had an increased WBSF of 0.20 and 0.35 kg, respectively, as a result of RH administration. Following 21 d postmortem aging, WBSF had reduced slightly, but the RH effects were not completely counteracted by aging.

Other influencers of tenderness of beef products should be examined to determine the impact of RH. The authors suggested that β -AA cause alterations to the proteolytic system through increases in calpastatin activity (Wheeler and Koohmaraie, 1992; Geesink et al., 1993) and a change to the muscle fiber profile (Vestergaard et al., 1994) of muscle tissue which is associated with the decrease in meat tenderness (Gruber et al. 2008). The system that plays a major role in postmortem meat tenderization is the calpain system, which consists of calcium dependent cysteine proteases that are responsible for degradation of key myofibrillar proteins such as titin and desmin (Lian et al., 2013). Today, little information exists about the effects of RH on the proteolytic system during postmortem aging of beef products. Strydom et al. (2009) examined samples of the LM for calpastatin, m-calpain, and μ -calpain activity. Ractopamine hydrochloride administration did not impact m- or μ -calpain activity, but calpastatin activity was increased when compared to control cattle. The correlation coefficient for calpastatin activity and WBSF increased as postmortem ageing time increased to 14 d postmortem. Calpastatin activity inhibits the calpain system resulting in less postmortem degradation of muscle tissue which

decreases the extent of tenderization of the myofibrillar fraction. Also, the total amount and solubility of collagen was similar for RH and control treatments. Martin et al. (2014) reported similar collagen percentage between non-RH fed and RH fed steers. More research is warranted for further explanation of the biochemical properties of muscle tissue from cattle administered RH.

Other quality measurements such as pH, water holding capacity, and moisture loss amounts have been reviewed to determine RH effects since these measures can impact final tenderness. Avendaño-Reyes et al. (2006) examined the WHC of beef steaks from steers supplemented RH. The measure of WHC was not influenced by treatment; however, the amount of drip loss displayed for the RH steaks was increased 1.8% when compared to control steaks. The amount of drip loss did not differ between control and RH supplemented steers, for this study. Martin et al. (2014) reported increased percent purge loss by 0.21% for steers subjected to RH when compared to the controls. Bohrer et al. (2014) measure loin pH and reported no difference between control and RH fed steers. Similar to the previous study, Holmer et al. (2007b) observed no difference in pH for strip loins from cull beef cows subjected to RH. Ultimate pH can have an impact on water holding measures in meat products during storage, thawing, or cooking. Quinn et al. (2008) analyzed the effects of RH on purge loss during retail display and cook loss after a cooking period for strip loin steaks from crossbred heifers. There were no observed differences between control and RH fed heifers for the two water holding measures. These authors also reported no difference in marbling score or quality grade. After cooking, Boler et al. (2012) measured the amount of cook loss between RH fed and control steers and displayed no difference in percentage lost, regardless of aging time. An impact on water

holding measures can have an effect on WBSF as moisture or juiciness is related to a pleasurable eating experience.

Zilpaterol Hydrochloride

The repartitioning effects of ZH are more pronounced for animal performance and carcass characteristics, which can cause more drastic impacts on meat tenderness. Strydom et al. (2009) observed the effect of ZH supplementation of LM steak tenderness after 2, 7, and 14 d of aging. When compared to control steaks, the ZH fed steers produced steaks that had increased WBSF at all d of aging. On d 2 of aging, the ZH steaks were approximately 1.3 kg tougher than the control steaks. Following 7 and 14 d of aging, the ZH steaks were approximately 1.9 kg tougher than the control steaks. This study did not display improvements in WBSF due to postmortem aging, as the ZH steaks were still drastically increased following 14 d of aging. Rathmann et al. (2009) observed the effect of ZH for different durations on strip loin WBSF when aged for 7, 14, or 21 d. Regardless of duration of ZH supplementation, steers fed ZH had increased WBSF after all three aging periods. There was a tendency for duration of ZH to respond linearly for WBSF after 7 d of aging, while WBSF after 21 d increased linearly as the duration of ZH increased as 20, 30, or 40 d supplementation increased WBSF by 0.9, 1.5, and 1.6 kg, respectively. There was also a tendency for d 21 WBSF to increase quadratically, or increase at a decreasing rate. Mehaffey et al. (2009) reported increased WBSF by 0.64 and 0.65 kg for Choice strip loin steaks aged to 14 d from Holstein steers fed ZH for 20 and 30 d. Scramlin et al. (2010) examined the effect of ZH supplementation on the WBSF measures of strip loin steaks. Initially after 3 d of aging, the ZH steaks were 2.23 kg tougher than the control steaks. Following 7 and 14 d of aging, the ZH steaks remained tougher than the control steaks by 2.15 and 1.73 kg, respectively. Even when aging was extended to 21 d the ZH steaks remained 1.24 kg tougher

than the control steaks. The authors noted that an increase of 0.5 kg in WBSF can be detected by consumers (Platter et al., 2003), so the steaks produced by ZH cattle would have a noticeable impact on tenderness for consumers. Claus et al. (2010) evaluated the usage of ZH at a concentration of 7.6 ppm for 0, 20, 30, or 40 d (**CON**; **ZH-20**; **ZH-30**; **ZH-40**) for beef steers prior to harvest. Strip loins were collected following commercial harvest procedures and chill period and were subjected to aging periods of 7, 14, or 21 d. Steaks from the ZH-20 cattle increased WBSF by 0.5 kg when compared to CON steaks. The ZH-30 and ZH-40 steaks had WBSF that were approximately 1.1 kg greater than CON steaks. After 7 d aging, the ZH-20 and CON steaks were similar, while the ZH-30 and ZH-40 shear force values remained approximate 0.5 kg greater than the CON steaks following 14 and 21 d of aging. The percentages of steaks that exceeded WBSF threshold limits from ZH treated animals was 51-79% after 7 DOA. After 21 DOA, there was no difference between any of the treatments for percentage of steaks that exceeded the threshold. Supplementing ZH for greater than 20 d can have lasting impacts on tenderness attributes for steaks subjected to postmortem aging. Brooks et al. (2010) observed the effect of ZH on meat tenderness of the strip loin from beef steers. The authors utilized a similar treatment scheme with the durations of ZH lasting 0, 20, 30, or 40 d and the steaks were aged 7, 14, or 21 d. Following 7 and 14 d of aging, all durations of ZH supplementation increased WBSF values for strip loin steaks when compared to the controls. After 21 d of aging, the steers supplemented ZH for 20 and 40 d produced steaks with similar WBSF as control steaks. The steers supplemented ZH for 30 d had similar WBSF to the 20 and 40 d ZH treatments but were increased by 0.39 kg when compared to the control steaks. Kellermeier et al. (2009) observed increased WBSF by 1.67 kg for steaks from steers supplemented ZH and aged for 7 d. Following 14 d of aging, the ZH steaks produced WBSF that were 1.29 kg greater than control steaks and

remained 1.4 kg tougher after 21 d of aging. Rodas-González et al. (2012) reported increased WBSF and SSF values for steaks from ZH supplemented steers on d 7, 14, 21, and 28 d postmortem aging. Postmortem aging decreased the WBSF values of all steaks, but after 28 d the ZH steaks were still 0.55 kg tougher than control steaks; however, the value of WBSF reported for ZH steaks at d 14 would be considered an acceptable level of tenderness as it was under the 4.2 kg guaranteed tenderness level. Similarly, Shook et al. (2009) reported increased WBSF for ZH steak on d 7, 14, and 21 of postmortem aging. On d 7, there was 46.2% of ZH steaks that exceeded the tenderness threshold of 4.5 kg, while only 17.4% of control steaks exceeded this level. After 21 d of aging, there was 10.8% of ZH steaks that remained above the tenderness threshold, while the control steaks only reported 1.6% of steaks over this threshold. Choi et al. (2013) measured the tenderness of LM steaks following 7 d of aging. For Hanwoo steers fed ZH, the WBSF was increased by 1.2 kg when compared to the non-ZH supplemented steers. Howard et al. (2014b) measured the SSF of top loin steaks from Holstein steers fed ZH, segregated by quality grade, and aged for 14 or 21 d. After 14 d of aging, all steaks from the three quality grades for ZH fed steers had increased SSF when compared to the controls, except for the ZH high Choice category which was similar to the control Select category. The Select ZH steaks had the greatest SSF when compared to the other treatments. Extending the aging period to 21 d allowed steaks from ZH fed steers to become similar to one another across the quality grade categories. The control low and high Choice steaks remained decreased for SSF when compared to the ZH steaks. Avendaño-Reyes et al. (2006) reported a 0.71 kg increase in shear force for steers fed ZH. Brooks et al. (2009) observed the effect of duration of ZH supplementation for 0, 20, 30 or 40 d and postmortem aging of 7, 14, and 21 on WBSF of strip loin steaks. There was no duration \times postmortem aging interactions, but duration and aging time impact the WBSF of

choice strip loin steaks. As duration of ZH supplementation increased, the WBSF increased linearly from 0 d to 40 d supplementation. Increasing the length of postmortem aging decreased the reported WBSF for all steaks, with a 17.6% decrease from d 7 to d 21 being observed for this study. Garmyn et al. (2010) reported increased WBSF of 0.82 kg for ZH fed Holstein steers. Another study conducted by Garmyn et al. (2011) analyzed WBSF of the *Longissimus lumborum* for crossbred steers subjected to ZH supplementation for 0 or 20 d. For this study, cattle administered ZH produced steaks with increased WBSF values. The steers not subjected to ZH had reduced WBSF by 1.15 kg after 7 d of aging. Following 28 d of aging, the ZH steaks remained tougher by 0.71 kg when compared to the control steaks. Extending the aging to 35 d reduced the difference between ZH steaks and control steaks to 0.29 kg but they remained different from one another due to ZH treatment. After aging to 28 d, the percentage of steaks that were below the consumer threshold was 99%. Korn et al. (2013) analyzed the effects of ZH on tenderness attributes of the LM. The authors reported shear force values for ZH steaks that were 1.63 kg greater after 7 DOA, 1.17 kg greater after 14 DOA, and 0.99 kg greater at 21 DOA when compared to steers not supplemented ZH. Martin et al. (2014) observed 0.43 kg increased WBSF for LM steaks aged 16 d from ZH fed steers when compared to the control steers. Following 23 d of aging, the ZH steaks remained 0.42 kg tougher than control steaks. Leheska et al. (2009) also examined the effect of ZH for durations of 20 or 40 d prior to slaughter on WBSF of LM steaks from steers and heifers. For both sex classes, there was no effect of ZH \times duration interaction or duration on WBSF. For steers supplemented ZH for 20 d, the steaks produced had increased WBSF of 0.72 kg. When supplemented for 40 d, the steers produced steaks that were 0.72 kg greater than control steaks. For the heifers, the steaks from heifers supplemented ZH for 20 and 40 d had increased WBSF of 0.84 and 0.77 kg, respectively. Holmer et al. (2009a) observed an

increase in WBSF by 0.8 and 1.2 kg for steaks from Holstein steers fed ZH for 20 or 30 d when compared to the not supplemented controls, respectively. Across 21 d of aging, the ZH steaks did not reach the same values as control steaks and finished with an increased WBSF of approximately 0.8 and 1.1 kg for the steers supplemented ZH for 20 or 30 d, respectively. Crossbred beef heifers were utilized in a study to determine the effect of DOF and impact of ZH on meat tenderness (Rathmann et al., 2012). Zilpaterol hydrochloride was supplemented at 0 or 8.33 ppm for 20 to 22 d with a withdrawal period of 3 to 5 d. Shear force values were increased with ZH supplementation after 7, 14, and 21 DOA. After 7 d of aging, the steaks from ZH fed heifers reported WBSF that were 0.78 kg greater than controls. Following 14 or 21 d of aging, the ZH steaks had 0.52 and 0.47 kg greater WBSF than the control steaks, respectively. The frequency of steaks shearing below 4.3 kg for WBSF was measured at 7, 14, and 21 d postmortem. Following 7 d of aging, the heifers subjected to ZH reported only 55% of steaks under the 4.3 kg mark for WBSF, while the control steaks reported 89% of steaks were below this level. After 14 and 21 d of aging, the ZH steaks reached a frequency of 83 and 90% below the 4.3 kg mark, while control steaks reached a frequency of 96 and 98%, respectively. McEvers et al. (2012) observed increased WBSF of 0.74, 0.53, and 0.34 kg after 7, 14, and 21 d of postmortem aging, respectively, for steers fed ZH when compared to steers not fed ZH. Bloomberg et al. (2013) reported increased WBSF of 0.45 kg for heifers administered ZH when compared to the controls. Voges et al. (2007) reported that the current industry averages for postmortem aging periods exceeds 21 d, which is long enough to mitigate the negative impacts ZH has on tenderness.

The impact of ZH on myofibrillar characteristics and collagen solubility has been explored due to their effects on meat tenderness. Fiber size has been correlated to WBSF or

tenderness measures and can be increased due to ZH supplementation. Kellermeier et al. (2009) reported increased WBSF of LM steaks from ZH fed steers as well as an increase of 5% in fiber diameter for ZH steers when compared to the controls. The proteolytic system was examined in order to explain the tenderness changes associated with using ZH. Strydom et al. (2009) reported increased calpastatin activity for ZH steaks when compared to control steaks. Also, the amount of total collagen was decreased by 17% for the ZH treatment, while the solubility was unaffected by treatment. Hilton et al. (2009) observed no changes to μ - or m- calpain and calpastatin activity for steers subjected to ZH supplementation. Rathmann et al. (2009) did not report a ZH effect for calpastatin mRNA abundance, but displayed a tendency for a linear decrease in calpastatin mRNA abundance as duration of ZH supplementation increased. In another study conducted by Korn et al. (2013), gene expression of μ -calpain and calpastatin were measured from the LM muscle of beef steers. For this study, no changes were observed for calpastatin activity, but there was a tendency for calpain activity to be less for ZH supplemented steers. The potential for decrease in calpain activity can influence the amount of postmortem degradation that occurs in the muscle. Vestergaard et al. (1994) displayed similar results when analyzing the effect of β -AA in young Friesian bulls. Rathmann et al. (2009) reported no difference in percentage of collagen for ZH and control steers. Weber et al. (2013) found increases in solubility of collagen tissue with no difference in the amount of total collagen, which the authors attributed this change to the development of newly synthesized collagen as animal growth improved with β -AA supplementation. Martin et al. (2014) observed a decreased collagen percentage by 0.12% for steers supplemented with ZH when compared to the control counterparts. Kellermeier et al. (2009) reported a decrease in the total amount of collagen by 29% for steers supplemented ZH during the finishing period. Growth-promoting technologies

can cause adaptations to the fractions that influence tenderness and create inconsistencies within beef products.

Other factors influencing tenderness measure that can be impacted by ZH include water holding capacity measures. Kellermeier et al. (2009) observed the effect of ZH on cook loss percentage during cooking of LM steaks. The authors reported no difference in the percent cook loss for steaks from ZH fed steers and control steers. The strip loin purge loss percentage was increased by 0.43% for steaks from ZH fed steers when compared to the control counterparts. Holmer et al. (2009a) examined the quality attributes of strip loins from Holstein steers. For purge loss, they reported no difference between treatment groups, as well as no difference in percent moisture. Thaw loss prior to cooking and cook loss were greater for ZH-fed cattle when compared to control cattle. The authors hypothesized that the thaw loss was minimal due to fat cover and short aging period; however, the increase in cook loss they believed was due to increased muscle fiber size and weakened support structure. Leheska et al. (2009) displayed no effect of ZH supplementation on the percent cook loss of strip loin steaks from both heifers and steers. Strydom et al. (2009) reported increased drip loss percentage of 1.29% for steaks from ZH supplemented steers when compared to the controls. Rathmann et al. (2009) observed an increase of 0.27% in strip loin purge loss for heifers subjected to ZH for 20 d when compared to control cattle. The authors determined this increase in purge loss was due to an increased amount of moisture because of increased protein and decreased carcass fat. Rathmann et al. (2012) observed an increase in purge loss percentage of 0.27% for strip loins from heifers subjected to ZH when compared to the controls. Garmyn et al. (2010) observed three types of moisture loss throughout the cooking process of strip loin steaks from Holstein steers subjected or not to ZH. Prior to cooking, the thaw loss percentage was measured and was not impacted due to ZH

supplementation. Following cooking, the cook loss percentage was measured and was unaffected by ZH. The last measure of moisture loss was chilling loss which was obtained following cooking and an overnight chill period. Chilling loss was unaffected by ZH supplementation for this study. Rodas-González et al. (2012) observed a 1.71% increased amount of purge loss, but cook loss was unaffected for steers supplemented ZH. Hope-Jones et al. (2012) also reported increased amounts of drip loss by 0.5% after 96 h of aging due to ZH supplementation for Bonsmara steers. Choi et al. (2013) observed an increase in cook loss by 1.8% and purge loss by 1.2% for Hanwoo steers supplemented ZH. The magnitude of change for meat quality measures should be considered when determining if ZH should be supplemented during the finishing phase for beef cattle. Martin et al. (2014) observed increased percent purge loss of 0.42% for ZH steaks when compared to the controls. When ZH is supplemented, the proportion of muscle is increased; and this can result in increased water as muscle fibers become larger. The increased amount of water within a muscle can be lost during multiple phases of processing meat products.

Chapter 3 - Effect of anabolic implants and zilpaterol-HCl on *Longissimus lumborum* muscle fiber morphometrics, collagen solubility, and cooked meat tenderness

Abstract

The objective of the study was to examine the effect of growth-promoting technologies (**GP**) on *Longissimus lumborum* steak tenderness, muscle fiber cross-sectional area (**CSA**), and collagen solubility. Crossbred feedlot heifers (n = 33; initial BW 464 ± 6 kg) were blocked by BW and assigned to one of three treatments: no GP (**CON**; n = 11); implant, no zilpaterol hydrochloride (**IMP**; n = 10); implant and zilpaterol hydrochloride (**COMBO**; n = 11). Heifers assigned to receive an implant were administered Component TE-200 on d 0 of the study, and the COMBO group received 8.3 ppm of zilpaterol hydrochloride for the final 21 d with a 3 d withdrawal period. Following harvest, strip loins were collected and fabricated into four roasts and aged for 3, 14, 21, or 35 d postmortem. Fiber type was determined by immunohistochemistry. After aging, objective tenderness and collagen solubility were measured. There was a treatment × day of aging (**DOA**) interaction for Warner-Bratzler shear force (**WBSF**; $P < 0.01$). At d 3 of aging, IMP and COMBO steaks had greater WBSF than CON steaks ($P < 0.01$). By d 14 of aging, the WBSF of IMP steaks was not different ($P = 0.21$) than CON steaks, but COMBO steaks had greater values than steaks of both treatments ($P < 0.02$). The COMBO steaks only remained tougher ($P = 0.04$) than the CON steaks following 35 DOA. Compared to CON muscles, IMP and COMBO type I, IIA, and IIX muscle fibers were larger ($P < 0.04$). Treatment, DOA, or the two-way interactions did not impact measures of total and insoluble collagen ($P > 0.31$). Soluble collagen amount tended to be affected ($P = 0.06$) by a treatment × DOA interaction which was due to COMBO muscle having more soluble collagen

that the other two treatments on d 21 of aging ($P < 0.02$). Correlation analysis indicated that type I, IIA, and IIX fiber CSA are positively correlated with WBSF at d 3 and 14 of aging ($P < 0.01$), but only type IIX fibers are correlated at d 21 and 35 of aging ($P < 0.03$). At these time periods, total and insoluble collagen became positively correlated with WBSF ($P < 0.01$). This would indicate that relationship between muscle fiber CSA and WBSF decreases during postmortem aging, while the association between WBSF and collagen characteristics strengthens. The use of GP negatively impacted meat tenderness primarily through increased muscle fiber CSA and not through altering collagen solubility.

Introduction

In beef cattle production, anabolic implants and beta-adrenergic agonists (**B-AA**) are the two most commonly employed growth-promoting technologies (**GP**). Duckett and Pratt (2014) conducted a comprehensive review of the literature and reported that implants can increase ADG by 18%, carcass weight by 5%, and ribeye area by 4%. Lean et al. (2014) conducted a meta-analysis of the available B-AA data and reported that Zilpaterol hydrochloride (**ZH**; Merck Animal Health, Summit, NJ) increases ADG by 0.15 kg, carcass weight by 15 kg, and ribeye area by 8 cm². While the positive impacts of both GP on feedlot performance and carcass measures are quite considerable, both technologies can have a negative impact on meat tenderness. Anabolic implants administered to heifers increase Warner-Bratzler shear force (**WBSF**) by 9% (Boles et al., 2009). Inclusion of ZH in the finishing diet of heifers elicits a greater response on WBSF by increasing values by 24% (Leheska et al., 2009). The exact biological mechanisms responsible for the reduction in tenderness is unknown.

Two major structural units of muscle elicit the majority of the influence on meat tenderness. The muscle fiber constitutes the major component of the myofibrillar fraction of tenderness and can elicit its influence based on fiber size (Crouse et al., 1991) or sarcomere length (Smulders et al., 1990). Collagen is the major component of the endomysium and perimysium, which both comprise the intramuscular connective tissue component that contribute to meat tenderness and texture (McCormick, 1999). Both GP increase muscle fiber hypertrophy (Kellermeier et al., 2009), but their effect on the collagen compartment is not well understood. Additionally, the ability of extended postmortem aging to reduce the impact that both structures elicit on meat tenderness is not known. The objective of the study was to examine the effect of two GP programs on LM muscle fiber cross-sectional area, collagen solubility, and their relation to meat tenderness after extended postmortem aging.

Materials and Methods

All experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee.

Animals

Crossbred feedlot heifers ($n = 33$; initial BW $464 \text{ kg} \pm 6$) were housed in individual pens located in an enclosed barn at the Kansas State University Beef Cattle Research Center. Each pen was 4.64 m^2 and contained metal pipe sidewalls, slatted floors for waste removal, an individual waterer, and a $75 \text{ cm} \times 51 \text{ cm}$ feed bunk. Animals were fed a similar diet (Table 3.1) and feed was delivered once daily to allow *ad libitum* access to feed. Bunks were managed to leave a minimum of 227 g of unconsumed feed per head daily.

Following a 10-d acclimation period, heifers were weighed, stratified from heaviest to lightest BW, and within each strata of four animals, heifers were randomly allocated to one of three treatments ($n = 11$ per treatment): no anabolic implant or ZH (**CON**), anabolic implant, no ZH (**IMP**), and anabolic implant and ZH (**COMBO**). Heifers designated to receive anabolic implants were administered a Component TE-200 implant (Elanco Animal Health, Greenfield, IN) in the left ear. Beginning on d 50 of the trial, ZH was included in the total mixed ration at 8.3 mg/kg dm for the COMBO heifers. These cattle were fed ZH for 21 d with a 3-d withdrawal period prior to harvest. One animal from the implant group was removed from study due to pregnancy.

Carcass Data and Loin Collection

On d 75 of the experiment, heifers were shipped 430 km to a commercial abattoir for harvest (Tyson Fresh Meats, Holcomb, KS). Final BW and HCW were recorded at harvest. Following a 36-h refrigeration period, marbling score, LM area, and 12th-rib s.c. fat thickness

were instrumentally collected (VBG 2000; e+v Technology GmbH & Co. KG, Oranienburg, Germany), and percent KPH was estimated by a trained university staff member. Strip loins (Institutional Meat Purchase Specifications 180) were removed from one side of the carcass from each heifer and transported back to the Kansas State University Meats Laboratory.

Sampling Procedures

Approximately 72 h postmortem, a 1.27-cm thick steak was removed from the anterior portion of the *Longissimus lumborum* (**LL**), beginning at the 13th rib of each loin, and perpendicular to the orientation of muscle fibers for immunohistochemistry. At the posterior end of the loin, ultimate pH was measured with a calibrated pH meter (model HI 99163; Hanna Instruments, Smithfield, RI). The remaining portion of each loin was fabricated into three 3.81-cm thick roasts and assigned in order of fabrication to 3, 14, 21, or 35 d of aging (**DOA**). After each aging period was completed, two 2.54-cm steaks were removed for collagen analysis and determination of Warner-Bratzler shear force. Following 21-d aging period, the remaining portion of the loin following fabrication was utilized to measure pH after postmortem aging.

Immunohistochemistry

The methods of Phelps et al. (2014a) were followed for immunohistochemical analysis. Briefly, a 1 cm × 1 cm × 1.27 cm sample was collected from the geometric center of the medial (**MED**), medial/lateral (**M/L**), and lateral (**LAT**) areas of the LL (Figure C.1). Samples were embedded and frozen in optimum cutting temperature (**OCT**) tissue freezing medium (Fisher Scientific, Pittsburgh, PA) using liquid nitrogen cooled, 2-methyl-butane (Fisher Scientific). Five micrometer cryosections were incubated for 1 h in a primary antibody cocktail consisting of anti-dystrophin (Thermo Scientific, Waltman, MA), anti-slow myosin heavy chain (BA-D5, Developmental Studies Hybridoma Bank, Iowa City, IA), and anti-myosin heavy chain all but

IIX (BF-35, Developmental Studies Hybridoma Bank). Following washing and incubation with the appropriate secondary antibodies (Alexa-Fluor 594, 633, and 488; Invitrogen, Grand Island, NY). Photomicrographs were captured using a Nikon Eclipse TI-U inverted microscope equipped with a DS-QiMC digital camera at a 10× working distance magnification (Nikon Instruments Inc., Melville, NY). Within each steak location, a minimum of 500 muscle fibers were analyzed for myosin heavy chain isoform and CSA. Fibers staining positive for BA-D5 and BF-35 were considered type I fibers. Fibers staining positive for BF-35, but negative for BA-D5 were considered type IIA fibers. All fibers staining negative for BA-D5 and BF-35 were considered type IIX fibers (Figure B.1).

Warner-Bratzler Shear Force

After each aging period, steaks were immediately subjected to cooking procedures. Cooking procedures for Warner-Bratzler shear force were conducted according to the Meat Cookery and Sensory Guidelines (AMSA, 1995). External fat was removed and steaks were weighed prior to cooking. A thermocouple wire (30-gauge copper and constantan; Omega Engineering, Stamford, CT) was inserted into the geometric center of each steak and internal temperature was monitored using a Doric Minitrend 205 monitor (VAS Engineering, San Francisco, CA). Steaks were cooked on Hamilton Beach Indoor/Outdoor grills (Hamilton Beach, Southern Pines, NC), turned once at 40°C and removed at 70°C. Steaks were allowed to cool for approximately 10 min, then weighed for calculation of the percent weight lost during cooking. After a 24-h chill period at 7±1°C, two 1.27-cm cores were removed from each anatomical area within the steak parallel to the muscle fiber orientation. Each core was sheared once through the center with a Warner-Bratzler shear head (100 kg compression load cell, crosshead speed of 250 mm/min) attached to an INSTRON testing machine (Model 5569; Instron, Canton, MA).

Collagen Solubility

After aging, each steak for collagen analysis was cut into the three areas of interest, frozen in liquid nitrogen, pulverized using a Waring blender (Waring Products Division, Harford, CT), and stored at -20°C until analysis. Hydroxyproline content was determined using the protocol adapted from Hill (1966) and Association of Official Analytical Chemists method 990.26 (AOAC, 2005). Three grams of pulverized tissue were mixed with 12 mL of Ringer's solution and incubated in a 77°C water bath for 80 min. Following incubation, samples were centrifuged at $2,250 \times g$ for 12 min at 20°C to separate insoluble and soluble fractions. Sulfuric acid was then added (3 mL concentrated sulfuric acid to soluble portion; 30 mL 3.5 M sulfuric acid to the insoluble portion) and fractions were incubated at 105°C for 16 h. After incubation, samples were removed and cooled for a minimum of 30 min., diluted with deionized water to 250 mL for the soluble fraction and 500 mL for the insoluble fraction, and then filtered with Whatman 541 filter paper (Fisher Scientific; Waltham, MA) into 15-mL glass test tubes and analyzed using a hydroxyproline assay the same day. Hydroxyproline determination was carried out following procedures outlined by Bergman and Loxley (1963) using a BioTek Eon spectrophotometer (Biotek Instruments Inc., Winooski, VT) to read absorbance at 558 nm. The spectrophotometer was calibrated using a distilled water blank sample, and readings were quantified by standard curves prepared each day of analysis. Total and fractional collagen contents were determined by multiplying hydroxyproline content of the soluble fraction by 7.25 and the insoluble fraction by 7.52 (Cross et al., 1973).

Statistical Analysis

Four statistical models, all utilizing animal as the experimental unit, were employed to analyze the data. All models were randomized complete block designs with initial BW as the

blocking factor. Feedlot performance, carcass characteristics, and d 21 moisture loss utilized GP treatment as the fixed effect and animal within block as the random effect. Cook loss and pH data were analyzed as a repeated measures experiment, utilizing GP treatment as the fixed effect and animal within block as the random effect. Day served as the repeated measure with animal/loin as the subject and compound symmetry as the covariance structure. Muscle fiber morphometric data was analyzed utilizing a 3×3 factorial arrangement with GP treatment and muscle location as the main effects and animal within block as the random effect. Collagen measures and WBSF were analyzed as a $3 \times 4 \times 3$ factorial arrangement with GP treatment, day of aging, and muscle location as the main effects and animal within block as the random effect. All data were analyzed utilizing the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC) and pairwise comparisons between the least square means of the factor levels comparisons, were computed using the PDIFF option of the LSMEANS statement. Correlations between WBSF and muscle characteristics were computed using the PROC CORR procedure of SAS. Differences were considered significant at $P \leq 0.05$ and tendencies at $P > 0.05$ and $P \leq 0.10$.

Results

Animal Performance and Carcass Characteristics

Individually fed animals were subjected to one of three treatments to determine the effect of GP on feedlot and carcass performance measures. At the beginning of the trial, all treatment groups did not differ ($P = 0.82$) in BW (Table 3.2). Throughout the 75-d feeding portion of the trial, GP treatment did not impact DMI and ADG ($P > 0.30$). Gain to feed ratio tended to be affected ($P = 0.06$) by GP treatment, but final BW was unaffected ($P = 0.48$) by treatment. The use of growth GP impacted ($P = 0.01$) HCW. Carcasses from the IMP treatment tended to weigh more ($P = 0.07$) than the CON carcasses, but did not weigh differently ($P = 0.11$) than COMBO

carcasses. Carcasses from the COMBO treatment were heavier ($P < 0.01$) than CON carcasses. Dressing percentage tended to be influenced ($P = 0.07$), while yield grade was impacted ($P = 0.03$) by GP treatment. The IMP and COMBO carcasses had decreased ($P < 0.04$) yield grade when compared to control carcasses, but were not different ($P = 0.63$) from each other. Similar to HCW, treatment did impact LM area and BF ($P < 0.04$). Implant carcasses tended to have larger ($P = 0.06$) LM area and reduced ($P = 0.09$) BF thickness when compared to CON carcasses. Zilpaterol treatment carcasses possessed larger ($P = 0.01$) LM area and less ($P = 0.01$) BF than CON carcasses. When compared to each other, COMBO and IMP carcasses did not differ in LM area or BF thickness ($P > 0.37$). Kidney, pelvic and heart fat percentage and IMPS 180 weight did not differ ($P > 0.11$) by treatment and marbling score tended to be decreased ($P = 0.09$) by GP treatment.

Moisture Retention and pH Measures

After 21 d of aging, moisture retention during the 21 d of aging was quantified on the remaining portion of the loin (Table 3.3). Treatment affected d 21 purge loss with loins from IMP and COMBO loins having increased moisture loss when compared to CON loins ($P < 0.02$). When compared to each other, IMP and COMBO loins did not differ ($P = 0.70$) in the amount of moisture lost during aging. The moisture retention issues exhibited by GP supplemented cattle were not observed during cooking. There were no treatment \times DOA interaction or treatment effects on cooking loss ($P > 0.87$), but DOA tended to impact ($P = 0.06$) cook loss. Steaks cooked on d 14 lost more moisture than steaks cooked on d 3 and 21 ($P < 0.03$). All other cook loss measures were not different from one another ($P > 0.12$). Ultimate pH was measured on d 3 and 21 and displayed a treatment \times DOA interaction ($P < 0.01$). As loins were aged from d 3 to d 21, the CON loins had a decrease ($P < 0.01$) in pH, while IMP and COMBO loins did not

experience a change in pH ($P > 0.24$). This resulted in IMP and COMBO loins having a greater pH than CON loins at d 21 of aging ($P < 0.04$), but their pH did not differ from each other ($P = 0.43$). Over all DOA, treatment did not affect ($P = 0.92$) pH; however, loin pH did drop ($P < 0.01$) from d 3 to d 21 of aging. It is also important to note, that the magnitude differences noted in this experiment are not large enough to impact meat quality.

Muscle Fiber Morphometrics

To explore biological factors influencing WBSF, immunohistochemical analysis was utilized to examine the effects of steak location and GP treatment on muscle fiber type and morphometrics (Table 3.5). Each of three myosin heavy chain isoforms was analyzed independently. There were no treatment \times location interactions for muscle fiber type percentages and associated fiber CSA ($P > 0.77$). Type I muscle fiber percentage was not influenced ($P = 0.16$) by treatment, but was influenced ($P = 0.03$) by location within steak. The MED location had less ($P < 0.01$) type I fibers than the M/L location, while the LAT location possessed the same percentage of type I fibers as the MED and M/L locations ($P > 0.14$). Both type IIA and IIX fiber percentages were influenced by location and treatment main effects ($P < 0.04$). The MED location possessed more type IIA fibers than the other two steak locations ($P < 0.01$), which did not differ ($P = 0.25$) from each other. The MED location possessed less ($P < 0.01$) type IIX fibers than the LAT location, but did not differ ($P = 0.18$) when compared to the M/L location. The LAT location also tended to have more ($P = 0.08$) type IIX fibers than the M/L location. The treatment effect on fiber distribution indicated that the IMP treatment possessed more type IIA fibers than the COMBO and CON treatments ($P < 0.04$), but COMBO and CON treatments did not differ ($P = 0.15$) from each other. The IMP treatment possessed less ($P < 0.01$) type IIX fibers than the CON group; however, the IMP and COMBO treatments did not

differ ($P = 0.39$) in type IIX fiber percentage. Muscles from COMBO heifers tended to possess fewer ($P = 0.06$) type IIX fibers than muscle from CON heifers.

Cross-sectional area data indicated that type I and IIA CSA was affected by steak location ($P < 0.04$), while there was no effect ($P = 0.40$) on type IIX fiber CSA. The LAT location had smaller ($P < 0.01$) type I and IIA fiber CSA when compared to the MED and M/L areas, which did not differ ($P > 0.78$) from each other. Type I and IIA fibers within the COMBO and IMP sections had larger muscle fiber CSA when compared to CON samples ($P < 0.04$), but were not different ($P = 0.14$) from each other. Type IIX fiber CSA from COMBO heifers were larger than IIX fibers from both IMP and the CON heifers ($P < 0.01$). When compared to one another, IMP type IIX CSA was greater ($P = 0.01$) than that of CON fibers.

Collagen Characteristics

Insoluble and soluble collagen fractions were measured in order to determine the effect of steak location, treatment, and aging on these fractions (Table 3.6). There were no treatment \times DOA \times location, treatment \times location, or DOA \times location interactions for amounts of soluble collagen measured ($P > 0.42$); however, there tended to be a treatment \times DOA interaction ($P = 0.07$). Day of aging influenced amount of collagen, with collagen solubility differing ($P < 0.01$) between d 14 and 21 and tending to differ ($P = 0.10$) d 3 and 21. All other day comparisons were not different from one another ($P > 0.13$). Location within steak also impacted collagen solubility, with M/L area possessing more soluble collagen than the MED and LAT areas ($P < 0.05$), and the MED area having more ($P = 0.02$) soluble collagen than the LAT area. Day of aging, treatment, location, and their two-way and three-way interactions did not affect total or insoluble collagen ($P > 0.11$).

Warner-Bratzler Shear Force

As expected, as DOA was increased, WBSF values of all steaks decreased ($P < 0.01$) and became more tender (Table 3.4). There was no treatment \times DOA \times location interaction ($P > 0.10$) for WBSF values. The treatment \times location interaction, treatment \times DOA, and DOA \times location interactions impacted objective tenderness ($P < 0.05$). For the treatment \times location interaction, the CON and IMP treatments were similar ($P = 0.11$) to one another, but were decreased ($P < 0.01$) when compared to the COMBO treatment at the MED location. At the M/L and LAT locations, the CON steaks did not differ ($P > 0.17$) from the IMP steaks, but were decreased ($P < 0.02$) when compared to the COMBO steaks. The IMP steaks did not differ ($P > 0.11$) from the COMBO steaks at the M/L and LAT locations. When analyzing treatment \times DOA interaction, at d 3 COMBO steaks had greater WBSF values than IMP and CON steaks ($P < 0.01$). Steaks from IMP treatment also had greater ($P = 0.02$) WBSF values when compared to CON steaks. After 14 d aging, the IMP and CON steaks did not differ ($P > 0.21$) from one another, but the WBSF for COMBO steaks continued to be greater than the CON and IMP treatments ($P < 0.02$). At d 21 of aging, WBSF values of steaks from all treatments did not differ ($P > 0.13$). Unlike the d 21 results, d 35 COMBO steaks were tougher ($P < 0.04$) than CON steaks; however, IMP steaks WBSF values did not differ from either of the other treatments ($P > 0.13$).

When considering DOA \times location interaction, the MED and M/L locations possessed similar ($P = 0.91$) WBSF at d 3 of aging. Also on this DOA, the WBSF values from the LAT location tended to be less when compared to the other two locations ($P < 0.08$). After 14 d of aging, MED cores had reduced WBSF values than cores from the M/L and LAT locations ($P < 0.02$), but cores from these locations did not differ ($P = 0.12$). Following 21 d aging, WBSF values of MED cores continued to be less ($P < 0.01$) than M/L cores, but there was no difference

($P = 0.17$) between MED and LAT cores. The M/L and LAT continued to maintain similar ($P = 0.13$) WBSF values on this DOA. After 35 d aging, the M/L location had greater ($P < 0.05$) WBSF values when compared to the other locations, which did not differ from one another ($P < 0.78$). Over all DOA, location of cores within the steak affected ($P < 0.01$) WBSF. The M/L had greater ($P < 0.01$) WBSF values when compared to the MED and LAT locations, but the MED and LAT locations were similar ($P > 0.32$) to each other. Treatment also impacted ($P < 0.01$) WBSF of steaks. Over the entire aging study, steaks from CON and IMP cattle had similar ($P = 0.15$) WBSF. When compared to COMBO steaks, the CON steaks had decreased ($P < 0.01$) WBSF values, while IMP steaks tended to have decreased ($P = 0.06$) WBSF values.

Correlation Coefficients

Pearson's correlation coefficients between WBSF and myofibrillar and collagen characteristics are displayed in Table 3.7. On day 3 of aging, the CSA of all fiber types were positively correlated with WBSF ($P < 0.01$), while all collagen characteristics were not correlated ($P > 0.10$). After 14 d of aging, CSA of all fiber types was still correlated ($P < 0.05$) to WBSF, but correlations were not as strong. All collagen measures were not correlated to WBSF ($P > 0.10$). At d 21 of aging, WBSF was not correlated to type I and IIA fiber CSA and soluble collagen content ($P > 0.14$). Type IIX fiber CSA was still correlated ($P = 0.03$) to WBSF at d 21 of aging and insoluble and total collagen content also became correlated ($P < 0.01$). These trends were maintained through d 35 of aging ($P < 0.05$), but the correlations were not as strong as d 21.

Discussion

Growth-Promoting Technologies Effects

Addition of GP improves feedlot performance and overall amount of lean muscle tissue (Schmidt and Olson, 2007). Currently in beef producers utilize several GP including, β -AA and

anabolic implants. Two β -AA options are approved for use in beef cattle, while the market currently possesses a multitude of anabolic implant options. In the current study, Component TE-200 or a combination of the implant and ZH were supplemented to finishing heifers. Over the 75-d feeding period, use of GP technologies did not improve feedlot performance measures, which is in contrast to the plethora of literature that documents drastic improvements in these measures. When examining studies that utilize implant strategies that consist of a similar hormone dosage (200 mg trenbolone acetate/20 mg estradiol) as the current study, heifer ADG and G:F can be increased by up to 50% and 31%, respectively (Herschler et al., 1995; Popp et al., 1997; Guiroy et al., 2002). In agreement with the current study, Garber et al. (1990) found that implanting heifers with Synovex-H did not affect G:F; however, ADG was improved by the implant. The addition of ZH to feedlot diets provides equally impressive results by increasing ADG and G:F by up to 18% and 25% , respectively (Montgomery et al., 2009a; Rathmann et al., 2012). In the current study, combining the implant regimen with ZH did not improve feedlot performance of the heifers when compared to controls; however, the magnitude of increase in ADG and G:F are similar to improvements reported in literature (22% and 18%, respectively). Therefore, it is possible that the lack of significance could be due to small animal numbers utilized in the study.

While GP regimens did not provide improvements in feedlot performance, advantages were demonstrated when examining carcass characteristics. In the current study, the IMP treatment HCW and LM area tended to increase by 2% and 14%, and increased yield grade by 28% when compared to CON carcasses, respectively. Garber et al. (1990) reported that implanting heifers did not increase HCW or yield grade, but did report a 6% increase in LM area. Other studies indicate that anabolic implants can increase both HCW and LM area modestly by

8% (Herschler et al., 1995; Popp et al., 1997). When ZH was added to the GP program and compared to the CON carcasses, HCW, yield grade, and LM area were increased by 5%, 33%, and 18%, respectively. The increase in HCW is comparable to other studies (Montgomery et al., 2009a; Robles-Estrada et al., 2009; Rathmann et al., 2012); however, the increase in LM area and yield grade are much greater than these studies, which may be due to the combination of GP utilized in the COMBO treatment. Montgomery et al. (2009a) found that heifers supplemented ZH without being administered anabolic implants had only a 9% increase in LM area and yield grade when compared to heifers not subjected to GP technologies. Both Robles-Estrada et al. (2009) and Rathmann et al. (2012) employed an anabolic implant in their control treatment and found that ZH can increase LM area and yield grade by only 6% and 9%, respectively. This is similar to the current study, but the difference between the COMBO and IMP treatments was not significant.

Beta-adrenergic agonists are commonly referred to as repartitioning agents due to their ability to redirect nutrients from adipose to muscle growth. Carcasses from ZH heifers had 35% less s.c. fat, but there was no effect on marbling score. The reduction in s.c. fat was much greater than the 5% reduction reported by Rathmann et al. (2009), but these authors also found no effect on marbling. In contrast, Montgomery et al. (2009b) reported that s.c. fat thickness was unaffected by ZH, but marbling score was reduced instead. In agreement with the ZH literature, the effect of implants varies from study to study. In their review of the literature, Duckett and Pratt (2014) stated that anabolic implants do not or minimally influence 12th rib s.c. fat thickness. Perry et al. (1991), Gerken et al. (1995), and Roeber et al. (2000) reported that various anabolic implants do not influence s.c. fat thickness. In the current study, s.c. fat thickness tended to be reduced by 25% in the IMP carcasses. Scheffler et al. (2003) indicated there may be a time of

administration dependent influence on marbling score, with the administration of 1 implant late in finishing having the largest detrimental effect. The current study's results contradicts this finding by there being no effect on marbling with a single implant administration.

In the current study, WBSF values were greater for all treatment groups when compared to the majority of the literature. It is hypothesized this is due to all steaks being subjected to cooking without freezing, which can occur during WBSF analysis (Shanks et al., 2002). In their review of the literature, Garmyn and Miller (2014) concluded that use of implants and ZH during finishing increases WBSF; however, retail studies would indicate these technologies elicit no adverse effects on objective or subjective tenderness measures. In the current study, the IMP treatment produced steaks that were tougher than CON steaks at d 3 postmortem. By d 14 postmortem, IMP WBSF values were similar to CON steaks, which would indicate aging ameliorates adverse effects of implants on tenderness. This is in agreement with Schneider et al. (2007), who reported that steaks from heifers implanted with trenbolone acetate/estradiol required at least 14 to 28 d of aging to achieve WBSF values that would be predicted to achieve satisfactory consumer acceptance levels. Platter et al. (2003) found that implanted heifers maintained greater WBSF values through 21 DOA. In agreement, Kerth et al. (2003) and Boles et al. (2009) also found that implanting heifers did not affect WBSF values at up to 21-DOA.

While the previous data indicate that negative effects of implants on objective meat tenderness can be removed by postmortem aging, the impact of ZH on meat tenderness is substantial. Scramlin et al. (2010) reported that 21 d of postmortem aging did not alleviate the negative effects ZH elicits on cooked meat tenderness. Leheska et al. (2009) also found that heifers supplemented the same dosage of ZH produced steaks that were 24% greater in WBSF values after 28 DOA. Other studies also indicate that aging up to 35 d postmortem will not

alleviate the negative impacts of ZH on objective tenderness (Brooks et al., 2009; Garmyn et al., 2011; Rathmann et al., 2012). In the current study, steaks from the COMBO treatment had greater WBSF values through 14 DOA, where they were 40% and 24% greater than CON and IMP steaks, respectively. At d 21 of aging and due to a large reduction in shear force from d 14, COMBO steak WBSF were similar to the other two treatments. At d 35 of aging and due to a slight elevation in the COMBO WBSF values, COMBO steaks were 20% tougher than CON steaks, which would agree with the previous literature that extended aging does not alleviate the negative effects of ZH on tenderness. Also of importance to note, the USDA has set a threshold for a guaranteed tender steak at a WBSF value of 4.4 kg (ASTM, 2011). For the current study, steaks from CON heifers began below this threshold, while it took IMP steaks 21 d, and COMBO steaks never broke this threshold.

Myofibrillar toughness and connective tissue are the two determinants of shear force values (Møller, 1980). Myofibrillar proteins, consists of the contractile and cytoskeletal proteins that are responsible for muscle contraction and structure, respectively (Aberle et al., 2003). The importance of muscle fiber CSA is demonstrated by the correlation analysis conducted in the current study. Previous research by Crouse et al. (1991) indicated that average muscle fiber size was correlated to shear force at d 1 and 3 of aging, but not correlated on d 6 or 14. Through d 14 of aging in the current study, CSA of all fiber types were correlated to WBSF; however, for the remaining two aging periods, the CSA of the smaller muscle fiber types (type I and IIA) were not correlated to objective tenderness. These findings would indicate that the size of muscle fibers are bigger influencers of tenderness during early postmortem aging would, but ultimately, size of IIX fibers are the main contributors to meat tenderness when meat is aged past 14 d.

In beef cattle, postnatal muscle hypertrophy only occurs through hypertrophy of the existing fibers formed during fetal development (Strickland et al., 1978). Kellermeier et al. (2009) conducted a similar study in steers and reported that average muscle fiber diameter was increased by 3% and 6% when anabolic implants and ZH were utilized, respectively. Fritsche et al. (2000) observed increased CSA for all fiber types for steers implanted with 120 mg trenbolone acetate and 28 mg estradiol when compared to non-implanted steers. While Rathmann et al. (2009) and Baxa et al. (2010) did not directly measure fiber CSA or diameter, they did indicate that increases in the mRNA expression of type IIX muscle fibers demonstrated that ZH was increasing protein synthesis in this isoform. Clancey et al. (1986) reported that steers implanted with an anabolic implant had larger α -Red (type IIA) and α -White (type IIX) than non-implanted steers. Utilizing another B-AA, clenbuterol, Miller et al. (1988) and Vestergaard et al. (1994) found the diameter of type II fibers were increased. Phelps et al. (2014a) reported that steers supplemented ractopamine-HCl tended to have increases in the CSA of type IIA fibers. These findings would indicate ZH preferentially increase muscle fiber CSA of type II fibers; however in the current study, all 3 muscle fiber types were increased by GP. Type I and IIA fiber CSA were increased by implants and ZH by a maximum of 21%. This is the first data indicating that GP, with a focus on ZH, increase the CSA of heifer type I fibers. This finding could be a function of the immunohistochemical method utilized, but Gonzalez et al. (2007) reported increases in type I fiber CSA of cull cows administered an anabolic implant with and without ractopamine-HCl. Administration of an anabolic implant increased type IIX fiber CSA by 10% when compared to CON fibers. When ZH was added to the GP regimen, type IIX CSA was increased by an additional 16%. Since the size of type IIX fibers are the only fiber type

morphological factor correlated with WBSF at d 35 of aging, the magnitude of the type IIX fiber response to a growth-promoting technology will determine its influence on tenderness.

Connective tissue or collagen is the second major tissue structure than can affect cooked meat tenderness. Correlation analysis indicated that as the type I and IIA muscle fiber CSA became less correlated to WBSF, the total and insoluble collagen content correlation increased. Thus, this would indicate that these two collagen measures become more important to tenderness as meat is aged longer. While not conclusively proven, B-AA stimulate muscle hypertrophy by an increase in protein synthesis, a reduction in protein degradation, or a combination of both (for review, see Mersmann, 1998). Calpastatin protease analysis would indicate that B-AA increase activity of the enzyme to reduce protein degradation (Kretchmar et al., 1990; Koohmaraie et al., 1991; Strydom et al., 2009). Therefore, it is not unreasonable to hypothesize that B-AA may also slow the rate of collagen degradation, which could encourage the formation of collagen crosslinks and reduce the solubility of collagen (McCormick, 1999). In the current study, treatment did not affect any collagen measures. In contrast, Kellermeier et al. (2009) reported that steers not administered GP had more total collagen than steers administered a combination of anabolic implants and ZH. The authors hypothesized the increase in muscle CSA in the GP treatments may have diluted the amount of total collagen present in the muscle. Strydom et al. (2009) also found that when ZH was added to an anabolic implant regimen, total collagen content was reduced, while soluble and insoluble collagen percentage was unaffected. Utilizing a less aggressive implant regimens, Calkins et al. (1986) and Faucitano et al. (2008) reported no implant effect on total or soluble collagen content and percent soluble collagen. Huck et al. (1991) also found collagen solubility was unaffected when more aggressive implants were administered to steers.

Effects of Location Within Steak

When the LL was partitioned into the three locations examined in the current study, Phelps et al. (2014b) indicated that the locations differ in muscle fiber type composition with the MED location possessing less type I and more type IIX fibers than the other locations. Therefore, a secondary objective of the study was to determine if the GP affected the locations of the LL muscle differently. The lack of treatment \times location interactions for all dependent variables of interest indicates that the GP treatments affected all locations within the LL equally; however, there are still some important locations to note. There was a location \times DOA interaction for WBSF. On d 3 of aging, WBSF values of the LAT portion of the loin was 0.4 kg less than the MED and M/L locations. As aging was advanced to d 14 of aging, the WBSF of the MED portion of the loin dropped quite extensively causing it to be reduced compared to the M/L and LAT locations by 0.79 kg and 0.48 kg, respectively. By d 35 of aging, the WBSF of the MED and M/L locations did not differ, while the M/L location was greater from the two locations by a maximum of 0.46 kg. Numerous studies have examined the effect of location within the cross-section of the LL on WBSF and the results are quite divergent amongst the studies. Several studies indicate that the LAT location of the steak possesses the greatest WBSF (Hedrick et al., 1969; Berry et al., 1993; Kerth et al., 2002; Derington et al., 2011). In agreement with the current study Crouse et al. (1989) and Janz et al. (2006) reported that the LAT location sheared with less force than the MED location. In further examining the Derington data, there is a trend that the M/L location of the steak increases in WBSF as the grade of the animal decreases, which could indicate why the M/L is the toughest portion of the steak.

In addition to the marbling influence, the different trajectories of the locations' aging patterns would indicate that there are inherent differences in the biology of the locations. In addition to having the smallest WBSF values at d 3, the LAT location also possesses the smallest

type I and IIA fibers. Since these fibers influence shear at this time point, this could partially explain the WBSF differences seen at d 3. As the meat is aged postmortem, the data in the current study does not adequately explain the differences in the aging patterns between the locations. There is no clear muscle fiber type distribution pattern that could aid in explaining differences and soluble collagen content of the locations are opposite of what one would expect for the WBSF/soluble collagen relationship; the LAT location contains the least soluble collagen, while the M/L location possesses the most. Therefore, other mechanisms, such as the calpain proteolytic system, may be responsible for the divergent aging patterns of the 3 locations.

Conclusion

Growth-promoting technologies impacted carcass measures and strip loin characteristics. The addition of implants and ZH improved lean muscle measures of LM area and strip loin weight, but also negatively impacted objective tenderness. Fiber CSA positively correlated with WBSF early on in the aging period, while insoluble and total collagen became correlated after 21 DOA. Location within the steak also impacted WBSF measures, as the M/L portion produced the greatest values when following 35 DOA. The difference in tenderness due to location were not explained by the correlation between collagen and fiber CSA area measures, which warrants further investigation of the location effect on objective tenderness.

Table 3.1 Diet composition (DM basis) for crossbred heifers subjected to three exogenous growth-promoting programs

Ingredient, %	Treatments		
	CON ¹	IMP ¹	COMBO ¹
Steam-flaked corn	52.70	52.70	52.70
Corn gluten feed	36.15	36.15	36.15
Ground alfalfa hay	3.87	3.87	3.86
Ground wheat straw	2.95	2.95	2.95
Mineral/vitamin supplement			
Ground limestone	1.55	1.55	1.55
Salt	0.29	0.29	0.29
Potassium chloride	0.20	0.20	0.20
Vitamin A 30,000 premix	0.006	0.006	0.006
Vitamin E 44,092 premix	0.09	0.09	0.09
Monensin and tylosin premix ²	2.16	2.16	2.16
Calculated nutrient composition			
NEm, Mcal/kg	0.95	0.95	0.95
NEg, Mcal/kg	0.65	0.65	0.65
ADF, %	9.00	9.00	9.00
NDF, %	21.46	21.46	21.46
CP, %	14.56	14.56	14.56
Ether extract, %	3.60	3.60	3.60
Ca, %	0.69	0.69	0.69
P, %	0.52	0.52	0.52
K, %	0.80	0.80	0.80
Vitamin A (added), IU/kg	0.98	0.98	0.98
Vitamin E (added), IU/kg	19.67	19.67	19.67

¹Crossbred feedlot heifers (n=33) were subjected to one of three treatments: no implant or zilpaterol hydrochloride (**CON**); Component TE-200 implant (Elanco Animal Health, Greenfield, IN) on d 0 of study, no zilpaterol hydrochloride (**IMP**); and Component TE-200 implant on d 0 of study and zilpaterol hydrochloride (Merck Animal Health, Summit, NJ) at 8.3 mg/kg of ZH from d 52 to d 72, followed by a 3 d withdrawal (**COMBO**).

²Formulated to provide 300 mg/day monensin and 90 mg/day tylosin (Elanco Animal Health) per animal in a ground corn carrier.

Table 3.2 Feedlot performance and carcass characteristics of crossbred heifers subjected to three exogenous growth-promoting programs

Item	Treatments			SEM	P-value
	CON ¹	IMP ¹	COMBO ¹		
Feedlot performance					
Initial body weight, kg	463.7	463.8	463.9	2.0	0.82
Final body weight, kg	530.9	541.8	541.9	6.6	0.31
Dry matter intake, kg	8.5	8.2	8.1	0.2	0.48
Average daily gain, kg	0.9	1.1	1.1	0.1	0.30
Gain:Feed	0.1070	0.1292	0.1278	0.0078	0.06
Carcass characteristics					
Hot carcass weight, kg	345.9 ^{a,x}	354.4 ^{a,b,y}	361.9 ^b	4.1	0.01
Dressing percentage, %	65.2	65.4	66.8	0.5	0.07
USDA yield grade	3.6 ^a	2.6 ^b	2.4 ^b	0.4	0.03
LM area, cm ²	78.2 ^{a,x}	88.8 ^{a,b,y}	92.2 ^b	3.7	0.03
12th-rib subcutaneous fat, cm	2.0 ^{a,x}	1.5 ^{a,b,y}	1.3 ^b	0.2	0.04
Kidney, pelvic, heart fat, %	2.5	2.4	2.4	0.1	0.59
Marbling score ²	610.9	534.0	560.9	23.5	0.09
Initial loin weight, kg	6.5	6.9	7.1	0.2	0.11

^{a,b}Means within a row with a different superscript are different ($P < 0.05$).

^{x,y}Means within a row with a different superscript tend to differ ($P < 0.10$).

¹Crossbred feedlot heifers (n=33) were subjected to one of three treatments: no implant or zilpaterol hydrochloride (**CON**); Component TE-200 implant (Elanco Animal Health, Greenfield, IN) on d 0 of study, no zilpaterol hydrochloride (**IMP**); and Component TE-200 implant on d 0 of study and zilpaterol hydrochloride (Merck Animal Health, Summit, NJ) at 8.3 mg/kg of ZH from d 52 to d 72, followed by a 3 d withdrawal (**COMBO**).

²Marbling scores: 500 = small00; 600 = modest00; 700 = moderate00.

Table 3.3 pH and moisture retention measures of subprimals and cooked steaks from the *Longissimus lumborum* of heifers subjected to three exogenous growth-promoting programs

	Treatments			SEM	P-value		
	CON ¹	IMP ¹	COMBO ¹		TRT ²	DOA ³	TRT × DOA
Purge loss ⁴ , %	0.51 ^a	0.84 ^b	0.90 ^b	0.14	0.02	--	--
Muscle pH					0.55	<0.01	<0.01
Day 3	5.65	5.62	5.62	0.02			
Day 21	5.55 ^a	5.62 ^b	5.60 ^b	0.02			
Cook loss ⁵ , %					0.92	0.06	0.87
Day 3	19.9	20.7	20.8	1.20			
Day 14	22.0	21.9	23.1	1.20			
Day 21	20.3	21.5	19.8	1.20			
Day 35	22.0	21.7	21.5	1.20			

^{a,b}Means within a row with a different superscript are different ($P < 0.05$).

¹Crossbred feedlot heifers (n=33) were subjected to one of three treatments: no implant or zilpaterol hydrochloride (**CON**); Component TE-200 implant (Elanco Animal Health, Greenfield, IN) on d 0 of study, no zilpaterol hydrochloride (**IMP**); and Component TE-200 implant on d 0 of study and zilpaterol hydrochloride (Merck Animal Health, Summit, NJ) at 8.3 mg/kg of ZH from d 52 to d 72, followed by a 3 d withdrawal (**COMBO**).

²TRT = Treatment.

³DOA = Day of aging.

⁴Purge loss calculated by $([\text{initial loin weight} - \text{final loin weight}] / \text{initial loin weight}) \times 100$. Initial and final (d 21) loin weight collected on posterior portion of the strip loin that remained following removal of steaks from the anterior end for Warner-Bratzler shear force.

⁵Cook loss calculated by $([\text{cooked weight} - \text{raw weight}] / \text{raw weight}) \times 100$.

Table 3.4 Warner-Bratzler shear force values from three locations within the *Longissimus lumborum* of heifers subjected to three exogenous growth-promoting programs¹⁻⁷

	Medial			Medial/Lateral			Lateral			SEM
	CON ⁸	IMP ⁸	COMBO ⁸	CON	IMP	COMBO	CON	IMP	COMBO	
Day 3	4.40	5.51	6.02	4.22	5.38	6.27	4.06	4.86	5.92	0.36
Day 14	3.36	4.12	5.18	4.43	4.99	5.61	4.20	4.34	5.55	0.36
Day 21	3.36	3.89	4.76	4.45	4.66	4.64	4.27	4.19	4.39	0.36
Day 35	3.68	3.75	5.07	4.37	4.52	4.99	4.03	4.33	4.32	0.36

¹Treatment × day of aging × location, $P = 0.25$.

²Treatment × day of aging, $P < 0.01$.

³Treatment × location, $P = 0.04$.

⁴Day of aging × location, $P = 0.04$.

⁵Treatment, $P < 0.01$.

⁶Day of aging, $P < 0.01$.

⁷Location, $P < 0.01$.

⁸Crossbred feedlot heifers (n=33) were subjected to one of three treatments: no implant or zilpaterol hydrochloride (**CON**); Component TE-200 implant (Elanco Animal Health, Greenfield, IN) on d 0 of study, no zilpaterol hydrochloride (**IMP**); and Component TE-200 implant on d 0 of study and zilpaterol hydrochloride (Merck Animal Health, Summit, NJ) at 8.3 mg/kg of ZH from d 52 to d 72, followed by a 3 d withdrawal (**COMBO**).

Table 3.5 Myosin heavy chain (**MHC**) distribution and cross-sectional area from three locations within the *Longissimus lumborum* of heifers subjected to three exogenous growth-promoting programs

	Medial			Medial/Lateral			Lateral			SEM
	CON ¹	IMP ¹	COMBO ¹	CON	IMP	COMBO	CON	IMP	COMBO	
MHC type I ²										
Percentage ²	28.7	27.8	29.6	30.3	30.8	32.1	30.2	28.5	30.5	1.5
Cross-sectional area ^{3,4} , μm^2	2,046	2,625	2,380	2,209	2,598	2,439	1,894	2,200	2,092	152
MHC type IIA ²										
Percentage ^{3,4}	43.6	50.2	46.8	39.6	44.3	42.4	38.2	43.7	39.0	2.8
Cross-sectional area ^{3,4} , μm^2	2,993	3,404	3,771	3,183	3,489	3,664	2,659	3,078	3,315	278
MHC type IIX ²										
Percentage ^{3,4}	27.7	22.1	23.6	30.1	24.9	25.5	31.7	27.8	30.6	3.0
Cross-sectional area ³ , μm^2	2,957	3,037	3,830	3,397	3,771	4,129	2,962	3,445	3,998	239

¹Crossbred feedlot heifers (n=33) were subjected to one of three treatments: no implant or zilpaterol hydrochloride (**CON**); Component TE-200 implant (Elanco Animal Health, Greenfield, IN) on d 0 of study, no zilpaterol hydrochloride (**IMP**); and Component TE-200 implant on d 0 of study and zilpaterol hydrochloride (Merck Animal Health, Summit, NJ) at 8.3 mg/kg of ZH from d 52 to d 72, followed by a 3 d withdrawal (**COMBO**).

²There were no treatment \times location interactions for MHC distribution or cross-sectional area ($P > 0.77$).

³Treatment, $P < 0.02$.

⁴Location, $P < 0.04$.

Table 3.6 Collagen characteristics from three locations within the LM from heifers subjected to three exogenous growth-promoting programs

Collagen, mg/g	Medial			Medial/Lateral			Lateral			SEM
	CON ¹	IMP ¹	COMBO ¹	CON	IMP	COMBO	CON	IMP	COMBO	
Soluble ^{2,3,4,5}										
Day 3	0.98	1.31	0.97	0.93	1.15	1.00	0.65	0.66	0.90	0.23
Day 14	0.78	0.81	0.56	0.70	1.53	1.08	0.66	0.63	0.62	0.23
Day 21	1.04	0.84	1.71	0.89	1.29	1.52	0.64	0.97	1.26	0.23
Day 35	0.63	1.13	1.02	1.40	1.22	0.90	0.78	0.83	0.89	0.23
Insoluble ⁵										
Day 3	6.94	5.04	6.32	5.15	4.18	4.66	4.99	5.00	5.25	0.94
Day 14	6.75	4.08	5.77	6.16	4.53	5.73	5.39	5.97	5.32	0.94
Day 21	4.72	6.76	4.82	4.95	5.13	5.42	5.90	4.62	6.76	0.94
Day 35	5.31	4.41	7.25	5.33	5.07	5.12	5.37	5.44	5.92	0.94
Total ⁵										
Day 3	7.92	6.35	7.33	6.08	5.33	5.71	5.64	5.66	6.17	1.03
Day 14	7.54	4.89	6.33	6.85	6.06	6.78	6.05	6.60	5.94	1.03
Day 21	5.74	7.60	6.53	5.84	6.42	6.94	6.54	5.59	8.02	1.03
Day 35	5.94	5.54	8.27	6.73	6.29	6.03	6.15	6.27	6.81	1.03

¹Crossbred feedlot heifers (n=33) were subjected to one of three treatments: no implant or zilpaterol hydrochloride (**CON**); Component TE-200 implant (Elanco Animal Health, Greenfield, IN) on d 0 of study, no zilpaterol hydrochloride (**IMP**); Component TE-200 implant on d 0 of study and zilpaterol hydrochloride (Merck Animal Health, Summit, NJ) at 8.3 mg/kg of ZH from d 52 to d 72, followed by a 3 d withdrawal (**COMBO**).²There were no treatment × day of aging × location interaction, treatment × location interaction, day of aging × location interaction, or treatment main effect ($P > 0.21$).

²Treatment × day of aging, $P < 0.06$

³Day of aging, $P < 0.03$.

⁴Location, $P < 0.01$.

⁵Treatment, day of aging, location, and their two- and three-way interactions were not significant ($P > 0.31$).

Table 3.7 Pearson’s correlation coefficients between Warner-Bratzler shear force and fiber cross-sectional area, soluble collagen, insoluble collagen, and total collagen for steaks aged 3, 14, 21, and 35 days postmortem from heifers subjected to three exogenous growth-promoting programs¹

Item	Warner-Bratzler shear force, kg			
	Day 3	Day 14	Day 21	Day 35
Cross-sectional area, μm^2				
Type I	0.51**	0.45**	0.26	0.19
Type IIA	0.51**	0.45*	0.27	0.25
Type IIX	0.64**	0.57**	0.37*	0.37*
Soluble collagen, mg/g	-0.22	0.11	0.34	0.05
Insoluble collagen, mg/g	-0.22	0.14	0.49**	0.23*
Total collagen, mg/g	-0.26	0.15	0.54**	0.30*

* $P < 0.05$.

** $P < 0.01$.

¹Crossbred feedlot heifers (n=33) were subjected to one of three treatments: control, no implant or zilpaterol hydrochloride; implant only, Component TE-200 implant (Elanco Animal Health, Greenfield, IN) on d 0 of study, no zilpaterol hydrochloride; and combination, Component TE-200 implant on d 0 of study and zilpaterol hydrochloride (Merck Animal Health, Summit, NJ) at 8.3 mg/kg of ZH from d 52 to d 72, followed by a 3 d withdrawal.

Chapter 4 - Effect of anabolic implants and ractopamine-HCl on *Longissimus lumborum* muscle fiber morphometrics, collagen solubility, and cooked meat tenderness

Abstract

The objective of this study was to examine the effect of growth-promoting technologies (**GP**) and extended aging on *Longissimus lumborum* muscle fiber cross-sectional area (**CSA**), collagen solubility, and their relationship to cooked meat tenderness. Two groups of crossbred feedlot heifers (n = 33, initial BW 430 ± 7 kg; n = 32, initial BW 466 ± 7 kg) were blocked by BW and assigned to one of three treatments consisting of: no implant or ractopamine hydrochloride (**CON**; n = 21); implant, no ractopamine hydrochloride (**IMP**; n = 22); implant and ractopamine hydrochloride (**COMBO**; n = 22). Heifers assigned to receive an implant were administered a Component TE-200 on d 0 of the study, and heifers in the COMBO group received 400 mg·head⁻¹·d⁻¹ for 28 (Group 1) or 29 d (Group 2) at the end of 90 (Group 1) or 106 d (Group 2) feeding period. Following harvest, strip loins were collected and further fabricated into five roasts for postmortem aging (**DOA**) periods of 2, 7, 14, 21, or 35 d. After aging, Warner-Bratzler shear force (**WBSF**), muscle fiber cross-sectional area (**CSA**), and collagen solubility were measured. There was no treatment × DOA interaction for WBSF ($P = 0.86$), but treatment and DOA impacted WBSF ($P < 0.01$). Over the entire aging study, COMBO steaks had greater ($P < 0.01$) shear force values when compared to the CON steaks. The IMP steaks tended to have decreased ($P = 0.07$) shear force when compared to the COMBO steaks, but did not differ ($P = 0.11$) from the CON steaks. The IMP and COMBO treatments had increased type IIA fiber CSA when compared to CON ($P < 0.01$). When compared to each other, the IMP and COMBO type IIA fiber CSA did not differ ($P = 0.76$). Type I and IIX fiber CSA tended to be

increased by IMP and COMBO treatments ($P < 0.10$). There was no treatment \times DOA interaction for amount of soluble, insoluble, and total collagen ($P > 0.98$). Collagen amounts were not impacted by GP treatment ($P > 0.72$), but DOA influenced ($P = 0.04$) the amount of soluble collagen. Fiber CSA positively correlated ($P < 0.05$) with WBSF only on d 2 of aging, while soluble collagen amount tended to negatively correlate with WBSF on d 7 and 14 if aging ($P < 0.10$). The addition of GP to feedlot heifer production increased carcass muscle yield, but resulted in reduced strip loin steak tenderness. Fiber CSA was increased, but collagen characteristics were not impacted due to GP.

Introduction

Growth-promoting technologies (**GP**) are important tools for improving efficiency and increasing lean muscle yields in feedlot cattle. Anabolic implants and beta-adrenergic agonists (**B-AA**) are the two most common GP utilized by producers. Aggressive combination implants containing trenbolone acetate (**TBA**) and estradiol (**E₂**) enhance feedlot performance and carcass characteristics and can yield the producer a profit of \$162.81 per head (for review, see Duckett and Pratt, 2014). Since zilpaterol-hydrochloride (**ZH**) was voluntarily removed from the marketplace, ractopamine-hydrochloride (**RH**) remains the sole B-AA available for producers to utilize. While not as potent as ZH, Lean et al. (2014) estimated that RH increases ADG by 0.19 kg/d and LM area by 1.84 cm². While RH may not influence these measures as strongly as ZH, the authors did note that RH increases Warner-Bratzler shear force (**WBSF**) by 0.2 kg.

Factors influencing meat tenderness and quality traits can impact the overall consumer acceptability of beef products (Lusk et al., 2001). Alterations to these myofibrillar or collagen characteristics during growth can have lasting impacts on meat tenderness. In a previous study, Ebarb (Chapter 3) reported that steaks from heifers administered anabolic implants or implants plus ZH had greater WBSF scores at d 3 of postmortem aging when compared to controls. As aging was extended, steaks from heifers administered implants only similar WBSF values than controls by d 14 of aging, while those administered the combination of GP never reached control shear values by d 35 of aging. Interestingly, the authors reported that the reduction in tenderness catalyzed by GP was due to their ability to increase muscle fiber CSA, while collagen solubility was not affected. The objective of this study was to examine the effect of GP and extended aging on skeletal muscle characteristics of the *Longissimus lumborum* (**LL**) and their relationship to cooked meat tenderness.

Material and Methods

Heifer Management

Two groups of crossbred feedlot heifers (group 1 $n = 33$, initial BW $430 \text{ kg} \pm 7$; group 2 $n = 32$, initial BW 466 ± 7) were housed in the same pens and barn described in Chapter 3. After a 10-d acclimation period for each replication, heifers were weighed, stratified from heaviest to lightest BW, and within each strata of 3 heifers, assigned to one of three treatments: no implant or RH (**CON**; $n = 21$); implant, no RH (**IMP**; $n = 22$); or implant and RH (**COMBO**; $n = 22$). Heifers in the IMP and OTA treatments were administered a Component TE-200 (Elanco Animal Health, Greenfield, IN) implant. Heifers in the COMBO treatment received $400 \text{ mg} \cdot \text{d}^{-1} \cdot \text{heifer}^{-1}$ of RH (Optaflexx; Elanco Animal Health) for 28 (Group 1) or 29 d (Group 2) before harvest. Heifers were fed experimental diets (Table 4.1) once daily for 90 and 106 d for group 1 and 2, respectively, to allow *ad libitum* access to feed. Bunks were managed to leave a minimum of 227 g of unconsumed feed per head daily. One animal from the CON treatment of group 2 was removed from study due to injury.

Harvest and Sampling Procedures

After feeding was completed, animals were shipped 275 km to a commercial abattoir (Creekstone Farms, Arkansas City, KS) for harvest. Animals were harvested under USDA inspection and carcasses were allowed to chill for 48 h before the collection of carcass measurements. Following a 48 h chill period, marbling score was collected by a USDA grader, while LM area and 12th-rib s.c. fat were instrumentally collected (VBG 2000; e+v Technology GmbH & Co. KG, Oranienburg, Germany). Strip loins (Institutional Meat Purchase Specifications 180) were collected and transported to the Kansas State University Meat Laboratory for further fabrication. A 1.27-cm thick steak was removed from the anterior portion

of the LL, beginning at the 13th rib of each loin, and was used for immunohistochemical analysis. Ultimate pH was measured using a meat pH meter (model HI 99163; Hanna Instruments, Smithfield, RI) at the geometric center of the loin. The loin was fabricated into five 5.08-cm thick roasts and randomly assigned to a postmortem aging period of 2, 7, 14, 21, or 35 d of aging (DOA). Roasts and the remaining portion of the loin were vacuum packaged and aged to assigned DOA at $5\pm 1^{\circ}\text{C}$. After aging, the roast was cut into one 2.54-cm steak for WBSF and one 2.54-cm steak for collagen solubility analysis. Steaks were stored in a blast cooler at -40°C until further analysis was completed. Following a 21-d aging period, the portion of the loin remaining after fabrication was utilized to measure d 21 pH.

Immunohistochemistry

The methods of Phelps et al. (2014a) were followed for immunohistochemical analysis. Briefly, a 1 cm \times 1 cm \times 1.27 cm sample was collected from the geometric center of the medial, medial/lateral, and lateral areas of the LL. Samples were embedded and frozen in optimum cutting temperature tissue freezing medium (Fisher Scientific, Pittsburgh, PA) using liquid nitrogen cooled, 2-methyl-butane (Fisher Scientific). Five micrometer cryosections were incubated in a primary antibody cocktail consisting of anti-dystrophin (Thermo Scientific, Waltman, MA), anti-slow myosin heavy chain (BA-D5, Developmental Studies Hybridoma Bank, Iowa City, IA), and anti-myosin heavy chain all but IIX (BF-35, Developmental Studies Hybridoma Bank). Following washing and incubation with the appropriate secondary antibodies, photomicrographs were captured using a Nikon Eclipse TI-U inverted microscope equipped with a DS-QiMC digital camera at a 10 \times working distance magnification (Nikon Instruments Inc., Melville, NY). Cross-sectional area of a minimum of 500 muscle fibers from each animal were analyzed by myosin heavy chain isoform. Muscle fiber isoform was determined by fibers

staining positive for BA-D5 and BF-35 were considered type I fibers. Fibers staining positive for BF-35, but negative for BA-D5 were considered type IIA fibers. Fibers staining negative for BA-D5 and BF-35 were considered type IIX fibers (Figure B.1). Within each fiber type of each heifer/loin, an average steak CSA was calculated from all three areas within the LL (Figure C.1).

Cooking and Warner-Bratzler Shear Force Procedures

Cooking procedures for WBSF were conducted according to the Meat Cookery and Sensory Guidelines (AMSA, 1995). Prior to cooking, frozen steaks were removed from their vacuum package, placed on a metal tray with an absorbent pad (Dri-Loc 50, Cyrovac Sealed Air Corporation, Duncun, SC), covered in plastic food wrap (Sysco Corporation, Houston, TX), and allowed to thaw for 24 h at $7\pm 1^{\circ}\text{C}$. After thawing, steaks were weighed and a thermocouple wire (30-gauge copper and constantan; Omega Engineering, Stamford, CT) was inserted into the geometric center of each steak. Internal temperature was monitored throughout the cooking period using a Doric Minitrend 205 monitor (VAS Engineering, San Francisco, CA). Steaks were cooked until the internal temperature at the geometric center reached 65°C on a Cuisinart Griddler (Cuisinart, Stamford, CT) set at 232°C . Once final temperature was reached, steaks were removed from heat, the off temperature was recorded, and then the peak temperature was taken when the steak had reached the greatest temperature following cooking. Steaks were then allowed to cool for approximately 10 min and a final weight was obtained for the calculation of percent moisture lost during cooking. After a 24-h chill period at $7\pm 1^{\circ}\text{C}$, six 1.27-cm cores were removed from across the steak parallel to the muscle fiber orientation. Each core was sheared once through the center and perpendicular to the muscle fiber orientation using a Warner-Bratzler shear head attached an INSTRON Universal Testing Machine (Model 5569;

Instron, Canton, MA) with a 100 kg compression load cell and a crosshead speed of 250 mm/min.

Collagen Solubility

After aging, each steak for collagen analysis was diced, frozen in liquid nitrogen, pulverized using a Waring blender (Waring Products Division, Hartford, CT), and stored at -20°C until analysis. Hydroxyproline content was determined using the protocol adapted from Hill (1966) and official Association of Official Analytical Chemists method 990.26 (AOAC, 2005). One gram of pulverized, freeze-dried tissue was mixed with 12 mL of Ringer's solution and incubated in a 77°C water bath for 80 min. Following incubation, samples were centrifuged at $2,250 \times g$ for 12 min at 20°C to separate the supernatant consisting of the soluble collagen from the insoluble pellet. The supernatant was decanted into a separate tube and 3 mL of Ringer's solution was added to the insoluble pellet and centrifuged again. Sulfuric acid was added (3 mL concentrated sulfuric acid to soluble portion; 30 mL 3.5 M sulfuric acid to the insoluble portion) and the fractions were incubated at 105°C for 16 h. Samples were removed and cooled for a minimum of 30 min. Once cooled, samples were diluted with deionized water to 250 mL for the soluble fraction and 500 mL for the insoluble fraction, mixed well, and filtered with Whatman 541 filter paper (Fisher Scientific; Waltham, MA) into 15-mL glass test. Hydroxyproline determination was carried out following the procedures outlined by Bergman and Loxley (1963) using a spectrophotometer (BioTek Eon; Biotek Instruments Inc., Winooski, VT) reading absorbance at 558 nm. The spectrophotometer was calibrated using a distilled water blank sample, and readings were quantified by standard curves prepared for each day of analysis. Total and fractional collagen content was determined by multiplying the hydroxyproline content of the soluble fraction by 7.25 and the insoluble fraction by 7.52 (Cross et al., 1973).

Statistical Analysis

The effect of replication was analyzed and no treatment \times replication interactions were present; therefore, data were pooled. Data were analyzed as a randomized complete block design utilizing animal as the experimental unit and initial BW as the blocking factor. Growth-promoting treatment served as the fixed effect and animal within initial BW block served as the random effect. Collagen solubility, WBSF, and pH were analyzed as a randomized complete block design with animal/loin as the subject. Peak temperature was utilized as a covariate when analyzing WBSF. All data were analyzed utilizing the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) and pairwise comparisons between the least square means of the factor levels comparisons, were computed using the PDIFF option of the LSMEANS statement. Differences were considered significant at $P \leq 0.05$ and tendencies at $P > 0.05$ and $P \leq 0.10$.

Results

Heifer Performance, Carcass Characteristics, and Loin Measures

To evaluate the feedlot and carcass response of feedlot heifers to common growth-promoting technologies, two groups of crossbred heifers were subjected to three exogenous growth-promoting programs (Table 4.2). Initial BW before RH supplementation did not differ ($P = 0.39$) due to IMP treatment. Following administration of the GP programs, final BW was not different ($P = 0.14$). Average daily gain, DMI, and G:F were not affected by GP treatment ($P > 0.11$).

Hot carcass weight tended to differ ($P = 0.09$) due to GP treatment; however, dressing percentage did not differ ($P = 0.41$). Carcasses from the IMP treatment tended to be heavier ($P = 0.07$) than CON carcasses. Carcasses from the COMBO treatment were heavier ($P = 0.05$) than CON carcasses, but did not differ ($P = 0.86$) from IMP carcasses. When evaluating the amount

of lean muscle deposition, GP treatment affected LM area ($P = 0.01$), with COMBO and IMP carcasses having larger LM area than CON carcasses ($P < 0.01$). Carcasses from COMBO and IMP treatments had similar ($P = 0.53$) LM area when compared to one another. Adipose measurements, including 12th-rib s.c. fat and marbling, were unaffected by GP treatment ($P > 0.45$). In agreement with LM area data, when IMPS 180 loins were fabricated from carcasses, weight was affected ($P = 0.05$) by GP treatment. Loins from COMBO and IMP carcasses were heavier compared to the CON loins ($P < 0.03$), but were not different ($P = 0.94$) from each other. Day-21 purge loss was not affected ($P = 0.49$) by GP treatment.

pH, Cook loss, and Warner-Bratzler Shear Force

There were no treatment \times DOA interactions for pH, cook loss, or WBSF ($P > 0.34$; Table 4.3). Growth-promoting treatment did not affect ($P = 0.98$) pH, but at 21 DOA, pH increased ($P = 0.01$). Percent cook loss was not affected by GP treatment or DOA ($P > 0.23$). Treatment and DOA influenced WBSF values ($P < 0.01$). All DOA aging comparisons differed from one another indicating that as postmortem aging time was increased, shear force values of steaks from all animals decreased ($P < 0.01$). Over the entire aging study, COMBO steaks had greater ($P < 0.01$) shear force values when compared to the CON steaks, but were not different ($P = 0.11$) from IMP steaks. Shear force of IMP steaks tended to be greater ($P < 0.07$) than CON steaks.

Immunohistochemistry

To explore effects of GP on muscle fiber morphometrics, immunohistochemistry was performed on muscle tissue samples within the LL. Each MHC isoform was analyzed independently of one another (Table 4.4). There was no GP treatment effect ($P = 0.48$) on the percentage of type IIA fibers. Growth-promoting treatment affected ($P = 0.04$) type I fiber

percentage and tended to affect ($P = 0.07$) type IIX fiber type percentage. Muscle from the LL of IMP cattle had an increased ($P < 0.01$) percentage of type I fibers when compared to the CON cattle. Percentage of type I fibers for the COMBO samples did not differ ($P = 0.41$) from IMP cattle, but tended to be greater ($P = 0.08$) compared to that observed for CON cattle. Muscle from the IMP treatment tended to have more ($P = 0.07$) type IIX fibers than CON muscle, while COMBO muscle possessed more ($P = 0.03$) type IIX fibers than CON muscle. There was no type IIX fiber percentage differences ($P = 0.72$) between IMP and COMBO muscles.

The CSA of type I and IIX fibers tended to be different due to GP treatment ($P < 0.10$). Compared to CON type I fiber CSA, IMP fibers tended to be bigger ($P = 0.07$), while COMBO fibers were bigger ($P = 0.05$). The CSA of IMP and COMBO type I fibers were not different ($P = 0.89$) from each other. Control muscle type IIX fiber CSA tended to be smaller ($P = 0.07$) than COMBO fibers and were also smaller ($P = 0.05$) than IMP muscle. Type IIX muscle fiber CSA did not differ ($P = 0.89$) between IMP and COMBO muscles. The CSA of type IIA fibers was impacted ($P < 0.01$) by GP treatment. The COMBO and IMP had increased type IIA CSA when compared to CON fibers ($P < 0.01$). When compared to each other, COMBO and IMP type IIA fiber CSA did not differ ($P = 0.76$).

Collagen Solubility

Fractions of soluble and insoluble connective tissue were separated and hydroxyproline content was measured using a colorimetric assay to determine the effect of treatment and postmortem aging on collagen solubility (Table 4.5). There were no treatment \times DOA interactions or treatment and DOA main effects for insoluble and total collagen content ($P > 0.84$). There were no treatment \times DOA interaction or treatment main effect for soluble collagen content ($P > 0.75$); however, there was a DOA effect ($P = 0.04$). Steaks aged for 2, 7, and 14-d

had less soluble collagen than steaks aged 35 d ($P < 0.03$). Additionally, d 7 and 14 tended to have less soluble collagen than d 21 ($P < 0.10$).

Correlation Coefficients

Pearson's correlation coefficients between WBSF and fiber CSA and collagen amounts are displayed in Table 4.6. On day 2 of aging, the CSA of type I and IIA fiber types were positively correlated with WBSF ($P < 0.05$), while there was a tendency for type IIX fiber CSA to correlate ($P = 0.09$). All collagen characteristics were not correlated to WBSF at 2 DOA ($P > 0.71$). For the remaining aging periods, the CSA of all fiber types were not correlated with WBSF ($P > 0.11$) except for a tendency for type I CSA to positively correlate ($P = 0.07$) on d 14. Soluble collagen was not correlated with WBSF on d 2, 21, and 35 ($P > 0.42$). On d 7 and 14, soluble collagen amount negatively correlated ($P < 0.08$) with WBSF. Collagen measures for insoluble and total amount were negatively correlated to WBSF on d 7 and 14 ($P < 0.05$), but not at 2 or 21 d ($P > 0.16$). At d 35 of aging, WBSF was negatively correlated to insoluble and total collagen content ($P < 0.01$).

Discussion

The use of GP in feedlot production can improve efficiency and increase carcass yields (Wileman et al., 2009; Lean et al., 2014); however, the current study demonstrated no effect on feedlot performance measures after employing the two GP treatments. Similarly, Ebarb (Chapter 3) reported no improvements to feedlot performance over controls when heifers were administered an anabolic implant only or combination treatment consisting of an anabolic implant and ZH supplementation. In contrast, some authors have reported improvements to ADG of up to 13% and feed efficiency by 11% for heifers receiving an implant containing TBA and E₂ (Cook et al., 2000; Cleale et al., 2012). Wagner et al. (2007) reported a 9.2% improvement in

feed conversion for heifers implanted with a combination implant compared to the non-implanted controls. Ractopamine hydrochloride studies display inconsistent results for feedlot efficiency measures in heifers. Sissom et al. (2007) reported that heifers administered implants and RH had improved ADG and G:F by 2% and 4%, respectively. Griffin et al. (2007) reported RH increased G:F by 4% and tended to increase ADG by 3%. After RH supplementation, Talton et al. (2014) observed similar results as the current study with no difference in feed efficiency measures, while Bryant et al. (2010) observed drastic improvements in ADG by 38% and G:F by 38%, but no differences in DMI. While the current study did not report statistically different feedlot performance measures, the differences in ADG and G:F are comparable to some of the previous larger pen studies. Therefore, the lack of improvement in performance measures for GP heifers in the current study could be a mechanism of the size of the study.

While no improvements in feedlot performance were observed during the feeding portion of the trial, carcass characteristics were impacted by GP treatment. Hot carcass weight tended to be affected by the use of GP, as the COMBO and IMP carcasses were 4% heavier than the CON carcasses. In chapter three, Ebarb reported that carcasses from heifers implanted with TBA and E₂ tended to be 2% heavier than CON carcasses. Similarly, Boles et al. (2009) administered TBA/ E₂ implants to feedlot heifers and reported increased HCW by 2% when compared to non-implanted heifers. Quinn et al. (2008) reported an increase in HCW by 2% for heifers that were not implanted but received RH during the finishing period. Ebarb (Chapter 3) observed 4% heavier carcasses for implanted heifers supplemented ZH during finishing when compared to the control heifers. For the current study, dressing percentage and yield grade were unaffected by GP treatment. In contrast, Ebarb (Chapter 3) reported a tendency for dressing percentage to be increased due to GP treatment, while yield grade was decreased by 28% and 33% for carcasses

from implanted heifers and implanted plus ZH fed heifers, respectively. Bruns et al. (2005) observed similar yield grade measures, but increased dressing percentage by 0.6% for carcasses from steers that received a combination implant when compared to the non-implanted steers. Herschler et al. (1995) reported no difference for yield grade and dressing percentage due to implant treatment. Talton et al. (2014) observed increased dressing percentage by 1.2% and no difference in yield grade for implanted heifers supplemented RH when compared to the non-RH supplemented implanted heifers.

As more nutrients are converted to lean muscle tissue, cattle supplemented with GP have a greater potential for increased lean muscle yields. This study demonstrated this mechanism with a 9 and 7.6% increase in LM area for COMBO and IMP loins, respectively, when compared to CON. The previous chapter (Ebarb, Chapter 3) observed a tendency for implanted heifers to have increased LM area by 11.9% when compared to the non-implanted controls. Cook et al. (2000) and Smith et al. (2007) observed an increase in LM area by as much as 18% after implanting heifers with a similar implant containing TBA and E₂. With RH supplementation, Griffin et al. (2009) reported increased REA. In contrast, Bryant et al. (2010) observed no difference in LM area for heifers subjected to RH. In Ebarb (Chapter 3), the implanted heifers supplemented ZH, instead of RH, had a 15.2% increase in LM area when compared to the controls. The current study also reported an increase for initial strip loin weight as the IMP and COMBO loins were 7% heavier than loins from control heifers. Kellermeier et al. (2009) reported increased strip loin yield by 0.19% for steers subjected to combination implants during finishing. In contrast, the heifers in Chapter 3 did not report increased strip loin weight when subjected to combination implants and ZH. Garmyn et al. (2014) reported increased strip loin weight by 3 and 7% for steers supplemented RH and ZH during the finishing period, respectively. The

improvements in LM area and strip loin weight demonstrate the effect of GP on increasing lean muscle yields, while there was no effect seen in feedlot performance.

The use of GP diverts nutrients toward lean muscle deposition rather than adipose tissue, which can have impacts on carcass adipose measurements (Duckett et al., 1999). In the current study, heifers did not display differences in 12th-rib s. c. fat or marbling scores when subjected to combination implants. In contrast, the implanted heifers in Chapter 3 tended to have less 12th-rib s. c. fat by 25% and tended to have decreased marbling score. Other early studies have displayed decreased marbling scores up to 15% for cattle that received a combination implant (Garber et al., 1990; Cecava and Hancock, 1994; Herschler et al., 1995). In agreement with the current study, some authors have also reported no change to FT or marbling score for cattle that were subjected to anabolic implants (DeHaan et al., 1990; Cook et al., 2000). The current study reported no differences in 12th-rib s. c. fat or marbling scores for heifers subjected to combination implants and RH. Similarly, Talton et al. (2014) and Quinn et al. (2008) observed no difference in 12th-rib subcutaneous fat or marbling score after supplementing heifers with RH. In contrast, the addition of ZH decreased the 12th-rib s. c. fat by 35%, but marbling scores were not changed for the implanted heifers in Ebarb (Chapter 3). In the current study, RH was supplemented for 28 d, which is only a portion of the supplementing time allowed for that product, and heifers only received one implant during growth. Subjecting cattle to multiple implants, a greater concentration of RH, or a more potent β -AA can increase the magnitude of impact on adipose measurements displayed, as the animals have a greater potential for lean deposition (Platter et al., 2003; Arp et al., 2014). Platter et al. (2003) reported increased marbling scores for steers that received one implant during finishing when compared to steers that received multiple implants during finishing. Arp et al. (2014) reported decreased marbling scores

for steers that received 300 mg RH or 6.8 mg/kg ZH when compared to the non-supplemented controls, while the steers that received 200 mg RH did not differ from the controls. Utilizing technologies that increase skeletal muscle deposition can negatively impact adipose measurements (Duckett et al., 1999; Maxwell et al., 2015).

The negative impact of GP on meat tenderness can be detrimental to consumers' acceptance of beef products. The lack of an interaction between treatment and DOA indicates that all steaks, regardless of treatment, aged at the same rate during postmortem aging. Across the entire aging study, the current study observed a 9 and 17% increase in WBSF values for the IMP and COMBO steaks, respectively, when compared to the CON steaks. Smith et al. (2007) reported increased WBSF by 18% for steaks aged to one day from steers subjected to an implant containing 200 mg TBA and 28 mg E₂. Other authors have reported no differences in shear force for cattle that did or did not receive an aggressive implant during growth (Schoonmaker et al., 2001; Barham et al., 2003). Ebarb (Chapter 3) observed that steaks from implanted heifers took 14 d to reach a WBSF values similar to controls; however, steaks from heifers implanted and fed zilpaterol did not reach control steak WBSF values after 35 d of aging. Gruber et al. (2008) reported increased WBSF for steers fed RH, while Quinn et al. (2008) observed no difference in shear force values of steaks from heifers subjected to RH. In the previous study, it is important to note that loins were aged 14 d prior to analysis of WBSF. The effects of GP on tenderness are more prominent prior to postmortem aging. This was observed in a study performed by Boler et al. (2012), as steaks from cattle fed RH and aged 4 d had 13% greater WBSF, but were not different after 7, 14, or 21 DOA. The current study agrees with previous literature that IMP steaks required approximately 7 DOA to be similar to CON steaks, while COMBO steaks required 21 DOA to be similar to CON steaks (Schneider et al., 2007; Quinn et al., 2008).

The current study observed an alteration to the type I fiber percentage as IMP muscle samples had a greater percentage of type I fibers when compared to the CON. This change was at the expense of type IIX fibers since there was a tendency for IMP muscles to have less IIX fibers, while CON had the greatest amount of IIX fibers. Ebarb (Chapter 3) reported increased percentage of type IIA fibers at the expense of type IIX fibers for heifers that received only an implant during finishing. Similarly, the CON muscles had the greatest amount of type IIX fibers. The difference in response for the two studies could be a mechanism of obtaining the cattle from different locations and feeding the cattle for a longer period of time in the current study (Thornton et al., 2012). Chung et al. (2012) observed no difference in the mRNA expression of the fiber types within the LM of steers that were subjected to TBA and E₂ implants when compared to nonimplanted cattle. Gonzalez et al. (2007) reported no difference in the percentage of type I and II fibers for cattle subjected to RH and TBA separately, yet when the two GP were combined the percentage of type II fibers was increased. Due to the authors' immunohistochemistry procedure, Gonzalez et al. (2007) were unable to detect the two type II isoforms in the LM; therefore, they were unable to detect shifts within type II fibers. When subjected to RH and combination implants, the COMBO muscles in the current study tended to have an increased percentage of type I fibers compared to the CON. Type IIA fiber percentage was not impacted by treatment and type IIX fiber percentage tended to be decreased for the COMBO treatment when compared to the CON. In contrast to the current study, Gonzalez et al. (2008) observed an increase in type IIA fiber percentage for RH supplemented cattle when compared to the control cattle.

Since the number of muscle fibers is fixed after birth, the growth of muscle tissue due to implants and β -AA is caused by an increase in CSA of muscle fibers (Strickland et al., 1978).

The influence of muscle fiber CSA on cooked meat tenderness was illustrated in Chapter 3, where all three fiber types were moderately correlated to WBSF through 14 DOA and type IIX fibers remained moderately correlated through 35 DOA. Chriki et al. (2012) reported an association between larger fiber CSA and reduced tenderness values for steaks aged to 14 d. In the current study, all three fiber types were positively correlated to WBSF only at d 2 of aging, but these associations were eliminated by postmortem aging. The rapid loss of association between muscle fiber CSA and WBSF in the current study could be due to the GP not stimulating as large of an increase in CSA as what was seen in Chapter 3. In Chapter 3, the GP treatments increased type I, IIA, and IIX fiber CSA by a maximum of 12%, 21%, and 28%, respectively. Growth-promoting technologies in the current study only increased type I, IIA, and IIX fiber CSA by 10%, 17%, and 11%, respectively. Similar to the current study, Fritsche et al. (2000) reported increased fiber CSA by 22% for implanted steers when compared to non-implanted steers. Gonzalez et al. (2007) displayed an increase in type I CSA and no change to type II fiber CSA in the LM muscle of culled beef cows supplemented RH. In a subsequent study utilizing younger-growing cattle, Gonzalez et al. (2010) reported no difference in type I and II fiber CSA for feedlot steers subjected to RH. The major difference between the results of Chapter 3 and the current study is the implant/ β -AA treatment increase in type IIX CSA. In Chapter 3, ZH was utilized as the β -AA and this caused an increase in type IIX CSA over the implant effect. These are the largest fibers in the muscle and had the strongest correlation to WBSF. Since RH does not elicit as potent of a growth response as ZH (Avenidaño-Reyes et al., 2006) and it did not increase type IIX fiber CSA over the implant fibers, this could be the reason for a lack of correlation on WBSF in the current study.

As skeletal muscle deposition is increased, the network of collagen is continuously remodeled as growth and skeletal muscle hypertrophy occur within the animal (Purslow, 2014). As a result, newly synthesized collagen is deposited in the muscle tissue to support the growing structure. Immature or recently synthesized collagen consists of divalent crosslinks which are heat soluble, while mature collagen consists of trivalent crosslink bonds and are associated with decreased heat solubility (Bailey and Light, 1989; McCormick, 2009). Growth-promoting treatment did not impact collagen measurements for this study. The previous chapter also reported no treatment effects for all of the collagen measures. Similar to the current study, Calkins et al. (1986) reported no change to total or soluble collagen with the use of zeranol implants. Strydom et al. (2009) observed no difference for amounts of total collagen and the solubility of collagen for RH and control treatments. Similarly, Martin et al. (2014) reported similar total collagen percentage between non-RH fed and RH fed steers. While the use of GP did not impact collagen solubility in the current study, extended postmortem aging influenced the solubility of collagen. Jeremiah and Martin (1981) reported increased collagen solubility by 12% for LD samples aged from 24 h to 20 d. In contrast, Pierson and Fox (1976) reported no effect of postmortem aging on collagen solubility. Further examination of the influence of postmortem aging on degradation of the connective tissue fraction is crucial to understanding the impact of collagen on aged beef products.

When observing the correlation between connective tissue and WBSF, most connective tissue measures displayed correlations after d 7 of aging. For soluble collagen on d 7 and 14, a decreased WBSF tended to be associated with an increased amount of soluble collagen. The negative correlation displayed on d 7, 14, and 35 for total and insoluble collagen conflicts with data presented in the previous chapter and several studies that relate decreased connective tissue

amount to improved tenderness at one postmortem aging period (Jeremiah and Martin, 1981; Renand et al., 2001; Rhee et al., 2004; Lepetit, 2007). Torrescano et al. (2003) reported a strong positive correlation between WBSF and total or insoluble collagen of raw steaks aged to approximately 24 h. For the current study, it is hypothesized that the conflicting results when compared to the Ebarb (Chapter 3) were a result of location within the strip loin. Prior to aging, the loin was randomized to have equal DOA assigned to the different cuts throughout the loin, because of the previously reported tenderness gradient within the LL (Kerth et al., 2002). When compared to a previous chapter, reported positive correlations between WBSF and total and insoluble collagen when the loin was not randomized prior to the aging period. When the loin was randomized, it created inconsistent aging curves for the different samples which may have caused conflicting results for the correlation analysis.

Conclusion

Feedlot heifers that were subjected to GP did not have improvements in feedlot performance, but displayed increased lean muscle yields typical of what is seen when utilizing these technologies. The use of implants alone or implants and RH resulted in increased WBSF values of strip loin steaks across the entire 35 d aging period. The negative impact of GP on meat tenderness can be attributed to the increase in fiber CSA, as they had no effect on collagen solubility measures. Correlations between WBSF values and muscle fiber CSA were not as strong or long lasting as Chapter 3. It is hypothesized this is due to a lack of the ability of the GP to increase CSA. This is especially evident in the inability of the COMBO regimen to increase the CSA of type IIX fibers.

Table 4.1 Diet composition (DM basis) for crossbred heifers subjected to three exogenous growth-promoting programs

Ingredient, %	Group 1 (n = 33)			Group 2 (n = 32)		
	Treatments ¹			Treatments ¹		
	CON	IMP	COMBO	CON	IMP	COMBO
Steam-flaked corn	57.91	57.91	57.12	57.79	57.79	57.28
Wet corn gluten feed	30.00	30.00	31.08	30.00	30.00	30.86
Ground alfalfa hay	8.00	8.00	7.76	8.00	8.00	7.82
Mineral/vitamin supplement						
Ground limestone	1.46	1.46	1.40	1.46	1.46	1.40
Salt	0.30	0.30	0.29	0.30	0.30	0.29
Potassium chloride	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin A	0.006	0.006	0.005	0.006	0.006	0.005
Vitamin E	0.04	0.04	0.04	0.04	0.04	0.04
Monensin and tylosin premix ²	2.18	2.18	2.18	2.18	2.18	2.18
Calculated nutrient composition						
NEm, Mcal/kg	2.11	2.11	2.11	2.11	2.11	2.11
NEg, Mcal/kg	1.45	1.45	1.45	1.45	1.45	1.45
ADF, %	8.36	8.36	8.38	8.36	8.36	8.38
NDF, %	19.44	19.44	19.61	19.41	19.44	19.58
CP, %	14.12	14.12	14.26	14.09	14.12	14.24
Ether extract, %	3.67	3.67	3.66	3.67	3.67	3.66
Ca, %	0.70	0.70	0.69	0.69	0.70	0.67
P, %	0.48	0.48	0.49	0.48	0.48	0.49
K, %	0.70	0.70	0.70	0.70	0.70	0.70
Vitamin A (added), IU/kg	2,200	2,200	2,112	2,200	2,200	2,112
Vitamin E (added), IU/kg	22	22	22	21	22	21

¹Crossbred heifers (group 1 n=33; group 2 n = 32) were subjected to one of three treatments: no implant or ractopamine hydrochloride (**CON**; Elanco Animal Health, Greenfield, IN); Component TE-200 implant (Elanco Animal Health) on d 0 of study, no ractopamine hydrochloride (**IMP**); and Component TE-200 implant on d 0 of study and ractopamine hydrochloride at 400 mg•d⁻¹•heifer⁻¹ 28 d for group 1 and 29 d for group 2 (**COMBO**).

²Formulated to provide 300 mg/day monensin and 90 mg/day tylosin (Elanco Animal Health) per animal in a ground corn carrier.

Table 4.2 Feedlot performance, carcass characteristics, and boneless strip loin (IMPS 180) characteristics for crossbred heifers subjected to three exogenous growth-promoting programs

	Treatments ¹			SEM	P-value
	CON	IMP	COMBO		
Feedlot Performance					
Initial BW, kg	472.4	486.1	483.2	7.4	0.39
Final BW, kg	512.9	530.2	531.2	7.2	0.14
ADG, kg/d	1.48	1.62	1.76	0.10	0.18
DMI, kg/d	9.25	9.18	9.00	0.30	0.83
Gain:feed	0.1580	0.1746	0.2116	0.0186	0.11
Carcass Characteristics					
HCW, kg	327.1	340.7	341.9	5.2	0.09
Dressing percentage, %	63.6	63.8	64.3	0.4	0.41
USDA yield grade	2.8	2.3	2.4	0.2	0.12
LM area, cm ²	84.2 ^a	91.2 ^b	92.6 ^b	0.3	0.01
12th-rib subcutaneous fat, cm	1.3	1.1	1.2	0.1	0.45
Marbling ²	519	503	519	20	0.80
Initial loin weight, kg	5.71 ^a	6.16 ^b	6.14 ^b	0.14	0.05
Purge loss ³ , %	3.97	4.37	3.40	0.59	0.49

^{a,b} Means within a row with a different superscript are different ($P < 0.05$).

¹Crossbred heifers (group 1 n = 33; group 2 n = 32) were subjected to one of three treatments: no implant or ractopamine hydrochloride (**CON**; Elanco Animal Health, Greenfield, IN); Component TE-200 implant (Elanco Animal Health) on d 0, no ractopamine hydrochloride (**IMP**); and Component TE-200 implant on d 0 and 400 mg·d⁻¹·heifer⁻¹ of ractopamine hydrochloride for 28 d (group 1) or 29 d (group 2) prior to slaughter (**COMBO**). Final body weights were 482 and 505 kg for group 1 and 2 heifers, respectively.

²USDA marbling scores: 400-499 = Slight; 500-599 = Small.

³Purge loss calculated by $([\text{initial loin weight} - \text{final loin weight}] / \text{initial loin weight}) \times 100$. Initial and final (d 21) loin weight collected on posterior portion of the strip loin that remained following removal of steaks from the anterior end for Warner-Bratzler shear force.

Table 4.3 Objective strip loin measurements from the *Longissimus lumborum* of crossbred heifers subjected to three growth-promoting programs and aged 2, 7, 14, 21, or 35 days

Objective measures	Treatments ¹			SEM	P-Value		
	CON	IMP	COMBO		Trt ²	DOA ³	Trt × DOA
Muscle pH ⁴							
Day 2	5.49	5.49	5.50	0.01	0.98	<0.01	0.56
Day 21	5.60	5.59	5.59	0.01			
Cook loss ⁵ , %							
Day 2	20.80	22.78	23.58	0.62	0.23	0.61	0.34
Day 7	22.36	22.60	22.24	0.62			
Day 14	21.90	22.23	22.32	0.62			
Day 21	22.02	22.73	22.49	0.62			
Day 35	22.60	22.70	23.33	0.62			
WBSF ⁶ , kg							
Day 2	5.10	5.54	6.03	0.22	<0.01	<0.01	0.86
Day 7	4.25	4.79	5.10	0.22			
Day 14	3.52	3.89	4.29	0.22			
Day 21	3.20	3.70	3.92	0.22			
Day 35	3.02	3.13	3.48	0.22			

¹Crossbred heifers (group 1 n = 33; group 2 n = 32) were subjected to one of three treatments: no implant or ractopamine hydrochloride (**CON**; Elanco Animal Health, Greenfield, IN); Component TE-200 implant (Elanco Animal Health) on d 0 of study, no ractopamine hydrochloride (**IMP**); and Component TE-200 implant on d 0 of study and ractopamine hydrochloride at 400 mg•d⁻¹•heifer⁻¹ 28 d for group 1 and 29 d for group 2 (**COMBO**). Final body weights were 482 and 505 kg for group 1 and 2 heifers, respectively.

²Trt = Treatment.

³DOA = Day of aging.

⁴pH was measured using a calibrated pH meter (model HI 99163; Hanna Instruments, Smithfield, RI) from the geometric center of the strip loin on day 2 and from the center of the remaining loin on day 21 of postmortem aging.

⁵Cook loss calculated using the equation [(final cooked weight – raw weight)/(raw weight)] × 100.

⁶WBSF = Warner-Bratzler shear force.

Table 4.4 Myosin heavy chain distribution and cross-sectional area of skeletal muscle fibers within the *Longissimus lumborum* of crossbred heifers subjected to three growth-promoting programs

	Treatments ¹			SEM	P-Value
	CON	IMP	COMBO		
Myosin heavy chain type I					
Percentage, %	27.5 ^{a,x}	31.0 ^b	29.9 ^{a,b,y}	1.0	0.04
Cross-sectional area, μm^2	2083	2279	2293	75	0.10
Myosin heavy chain type IIA					
Percentage, %	42.8	43.8	45.8	1.7	0.45
Cross-sectional area, μm^2	3004 ^a	3541 ^b	3486 ^b	128	<0.01
Myosin heavy chain type IIX					
Percentage, %	29.7	25.2	24.3	1.8	0.07
Cross-sectional area, μm^2	3750	4193	4164	161	0.10

^{a,b}Means within a row with a different superscript are different ($P < 0.05$).

^{x,y}Means within a row with a different superscript tend to differ ($P < 0.10$).

¹Crossbred heifers (group 1 n = 33; group 2 n = 32) were subjected to one of three treatments: no implant or ractopamine hydrochloride (**CON**; Elanco Animal Health, Greenfield, IN); Component TE-200 implant (Elanco Animal Health) on d 0 of study, no ractopamine hydrochloride (**IMP**); and Component TE-200 implant on d 0 of study and ractopamine hydrochloride at $400 \text{ mg} \cdot \text{d}^{-1} \cdot \text{heifer}^{-1}$ 28 d for group 1 and 29 d for group 2 (**COMBO**). Final body weights were 482 and 505 kg for group 1 and 2 heifers, respectively.

Table 4.5 Collagen characteristics of strip loin steaks from crossbred heifers subjected to three exogenous growth-promoting programs and aged 2, 7, 14, 21, and 35 days

Item	Treatments ¹			SEM	P-Value		
	CON	IMP	COMBO		Trt ²	DOA ³	Trt × DOA
Soluble collagen, mg/g							
Day 2	1.00	0.98	0.94	0.06	0.75	0.04	1.00
Day 7	0.99	0.94	0.93	0.06			
Day 14	0.98	0.95	0.95	0.06			
Day 21	1.06	1.00	1.03	0.06			
Day 35	1.09	1.06	1.05	0.06			
Insoluble collagen, mg/g							
Day 2	7.28	7.19	7.16	0.32	0.84	0.86	0.98
Day 7	7.21	7.47	7.06	0.32			
Day 14	7.17	7.25	7.00	0.32			
Day 21	7.30	7.26	6.99	0.32			
Day 35	7.08	7.15	7.05	0.32			
Total collagen, mg/g							
Day 2	8.29	8.18	8.10	0.36	0.84	0.97	0.99
Day 7	8.20	8.42	8.00	0.36			
Day 14	8.15	8.20	7.95	0.36			
Day 21	8.36	8.27	8.02	0.36			
Day 35	8.17	8.21	8.10	0.36			

¹Crossbred heifers (group 1 n = 33; group 2 n = 32) were subjected to one of three treatments: no implant or ractopamine hydrochloride (**CON**; Elanco Animal Health, Greenfield, IN); Component TE-200 implant (Elanco Animal Health) on d 0 of study, no ractopamine hydrochloride (**IMP**); and Component TE-200 implant on d 0 of study and ractopamine hydrochloride at 400 mg•d⁻¹•heifer⁻¹ 28 d for group 1 and 29 d for group 2 (**COMBO**). Final body weights were 482 and 505 kg for group 1 and 2 heifers, respectively.

²Trt = Treatment.

³DOA = Day of aging.

Table 4.6 Pearson's correlation coefficients between Warner-Bratzler shear force and fiber cross-sectional area, soluble collagen, insoluble collagen, and total collagen for steaks aged 2, 7, 14, 21, and 35 days postmortem from heifers subjected to three exogenous growth-promoting programs¹

Item	Warner-Bratzler shear force, kg				
	Day 2	Day 7	Day 14	Day 21	Day 35
Cross-sectional area, μm^2					
Type I	0.28**	0.17	0.23*	-0.04	-0.11
Type IIA	0.24**	0.13	0.20	0.10	-0.01
Type IIX	0.21*	0.09	0.20	0.12	-0.03
Soluble collagen, mg/g	0.05	-0.24*	-0.23*	0.06	-0.11
Insoluble collagen, mg/g	-0.01	-0.34**	-0.33**	-0.23*	-0.40***
Total collagen, mg/g	-0.05	-0.33**	-0.33**	-0.19	-0.39***

* $P < 0.10$.

** $P < 0.05$.

*** $P < 0.01$.

¹Crossbred heifers (group 1 n = 33; group 2 n = 32) were subjected to one of three treatments: control, no implant or ractopamine hydrochloride (Elanco Animal Health, Greenfield, IN); implant only, Component TE-200 implant (Elanco Animal Health) on d 0, no ractopamine hydrochloride; and combination, Component TE-200 implant on d 0 and 400 mg•d⁻¹•heifer⁻¹ of ractopamine hydrochloride for 28 d (group 1) or 29 d (group 2) prior to slaughter. Final body weights were 482 and 505 kg for group 1 and 2 heifers, respectively.

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Appendix A - Immunofluorescence staining protocol: dystrophin, BF-35, BAD5 on bovine muscle cryosections

Blocking Solution

10% Horse serum (HS)/0.2% TritonX-100 in PBS (pH of 7.4)

Primary Antibodies

- 1.) Dystrophin (Prod#PA137587 Thermo Fisher)
 - a. Pierce Anti-dystrophin rabbit polyclonal
 - b. Dilution of 1:500
- 2.) BF-35 (mouse IgG1 DSHB BF 35)
 - a. Myosin heavy chain all but 2X
 - b. Dilution of 1:10
- 3.) BAD5 (mouse IgG2b DSHB BAD5)
 - a. Myosin heavy chain type 1
 - b. Dilution of 1:10

Secondary Antibodies

- 1.) Alexa-Fluor 488 goat anti-mouse IgG1 (Invitrogen Cat# A-21121)
 - a. Dilution of 1:1000
- 2.) Alexa-Fluor 633 goat anti-mouse IgG2b (Invitrogen Cat# A-21146)
 - a. Dilution of 1:1000
- 3.) Alexa-Fluor 594 goat anti-rabbit H&L (Invitrogen Cat# A-11012)
 - a. Dilution of 1:1000
- 4.) Hoescht (33342)
 - a. Dilution of 1:1000

Staining Procedure

- 1.) Use PAP pen to make a hydrophobic ring around the edge of each slide while the slide is dry.
- 2.) Incubate cultures with blocking solution (100 μ L per section) for 30 min at room temperature to block non-specific antigen binding
 - a. Use a tip box that has the top wrapped in foil and a very wet paper towel in the bottom to provide the humidity for all the steps where the volume per slide is minimal.
- 3.) Remove blocking solution from each slide using a pipette tip in the corner of the slide
- 4.) Add primary antibody solution (100 μ L per section) and incubate at room temperature in a humidified box for 1 h
 - a. Primary antibodies can be combined into a single solution.
 - b. Remember to account for the volume of both antibodies in your calculations.
- 5.) Rinse with PBS for 5 m
 - a. Repeat this step three times.
- 6.) Add secondary antibodies and DAPI solution (100 μ L per section) and incubate at room temperature in a humidified box for 30 m
 - a. Protect slides from light for the remainder of the procedures.
 - b. Secondary antibodies and DAPI can be combined into a single solution
- 7.) Rinse with PBS for 5 m
 - a. Repeat this step three times
- 8.) Coverslip with 9:1 glycerol/PBS solution.
- 9.) Let slides dry sufficiently

- 10.) Fingernail polish the edges of the slide to seal it.
 - a. Once dry, the slides can be stored at room temperature for 7 to 14 d.

Appendix B - Representative photomicrographs of immunohistological staining pattern of beef *Longissimus lumborum* muscle.

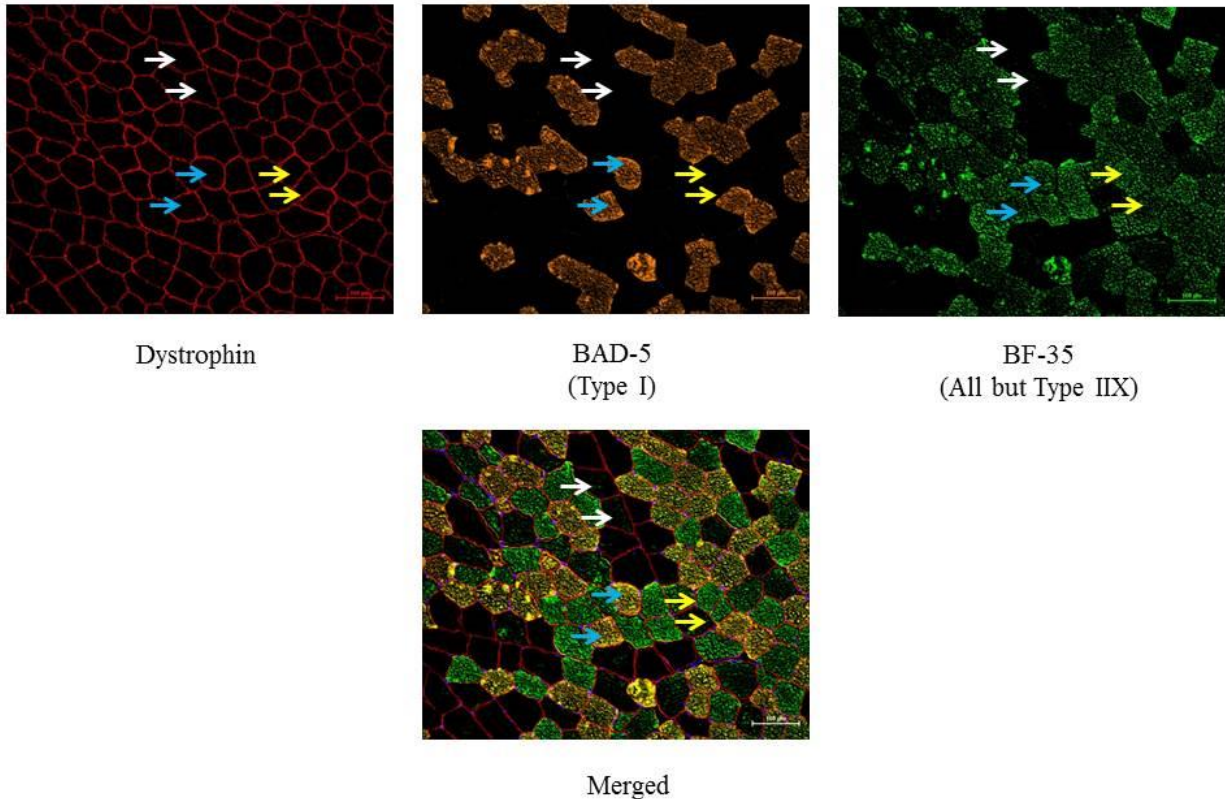


Figure B.1 Representative photomicrographs of immunohistological staining pattern of beef *Longissimus lumborum* muscle. Fibers that stained positive for the BA-D5 antibody were categorized as type I fibers (Blue arrows). Fibers that stained positive for BF-35, but were negative for BA-D5 were categorized as type IIA fibers (Yellow arrows). All fibers that were negative for the BF-35 antibody were categorized as type IIX fibers (White arrows; Moreno-Sanchez et al., 2008; Schiaffino et al., 1989). Scale bars = 100.

Appendix C - Representative photograph depicting the orientation of a strip loin steak as it was presented on the polystyrene foam tray.



Figure C.1 Representative photograph depicting the orientation of a strip loin steak as it was presented on the polystyrene foam tray. The steak was presented with the posterior portion of the cut facing up and the medial portion of the steak placed on the left side of the tray. For spectral color readings, readings were taken on the medial (**MED**), medial/lateral (**M/L**), and lateral (**LAT**) portions of the steak. These values were averaged to calculate the average spectral readings.

Appendix D - Hydroxyproline determination as an estimate of collagen (insoluble and soluble) in meat

Modified from:

AOCA. 2005. Official method 990.26. 18th ed. W. Horwitz and G.W. Latimer.

Hill, F. 1966. The solubility of intramuscular collagen in meat animals of various ages. J. Food Sci. 31:161-166.

A. Sample Preparation: Freeze Drying

- 1.) Freeze sample in liquid nitrogen and pulverize using Wharing blenders. Store in whirl pak bag in -20 until further analysis.
- 2.) Label, weigh, and record the weight of an empty weigh boat
- 3.) Place the minced sample in the weigh boat and record the weight of the wet sample plus the weigh boat.
- 4.) Place samples in a rack and remove caps. Follow instructions on the freeze drier and freeze solid (It usually takes 1 to 2 days)
- 5.) When samples are dry, remove from the freeze drier and move to desiccator. Weigh dry sample plus weight boat; record the weight as soon as possible.

B. Extraction/Hydrolysis

Prior to these steps, make sure you have enough supplies and reagents to complete all samples for the extraction group. Also, pre-label tubes for soluble and insoluble fractions to make the process more efficient.

Reagents:

3.5 M Sulfuric acid:

In a 2-L volumetric flask add 750 mL of MQ H₂O. Slowly add, with stirring, 375 mL concentrated Sulfuric acid. Cool to room temperature, and dilute to volume with MQ H₂O Mix and store in a labeled glass bottle at room temperature for up to 4 months.

¼ Strength Ringer's Solution:

In a 1000 mL beaker filled with 750 mL MQ H₂O, dissolve:

2.25g NaCl

0.1050g KCl

0.12 g CaCl₂·6H₂O) OR 0.0610g CaCl₂ anhydrous

0.05 Sodium bicarbonate

Check the pH. Adjust the pH to 7.0 ± 0.2 with 1.0 M HCl or 1.0 M NaOH. Transfer to 1-L volumetric flask and bring up to 1 L with MQ H₂O. Mix, and transfer to a labeled plastic bottle. Store at 4°C for up to 1 month.

Protocol:

- 1.) Preheat water bath to 77°C
- 2.) Weigh out 1 g of freeze-dried pulverized sample (to the nearest 0.01 g) into a 25mmx150mm glass Teflon-lined screw-cap tube and record weight
- 3.) Add 12 mL of ¼ Strength Ringer's Solution, place in water bath set to 77°C and incubate for 80 min while shaking
- 4.) Remove the tube from the water bath, and centrifuge at 3000 rpm for 12 min at 20°C
- 5.) Pour supernatant off in a tall-glass tube (Be careful not to allow particles from the insoluble pellet to go into the supernatant)

- 6.) Pipet 3 mL of $\frac{1}{4}$ Strength Ringer's Solution into the tube with the pellet
- 7.) Re-suspend the pellet by vortexing. Centrifuge at 3000 rpm for 12 mins at 20°C.
- 8.) Transfer supernatant to the tall-glass tube.
- 9.) To the tube with the supernatant, pipet 3.0 mL of concentrated H₂SO₄, cap tightly, and shake carefully to mix solution
- 10.) To the tube with the insoluble pellet, pipet 30 mL of 3.5 M H₂SO₄, cap tightly and shake carefully but forceful enough to break up the pellet at the bottom.
- 11.) Place all tubes in a **metal or autoclavable** rack and put in a drying oven set to 105°C for 16 to 20 h. Be consistent with hydrolysis times!
- 12.) After incubation in drying oven, remove samples from the oven and allow them to cool down for approx. 30 min before proceeding.
- 13.) Transfer the insoluble hydrolysate to a 500-mL volumetric flask, rinse tube and pour into flask with filtered DI H₂O then bring up to volume using filtered DI water **OR** Transfer the soluble hydrolysate to a 250-mL volumetric flask, rinse tube and pour into flask with filtered DI H₂O, then bring up to volume using filtered DI H₂O.
- 14.) Mix thoroughly by pouring solution into a beaker and stir using a stir bar and stir plate for approx. 2 min (Time it takes to bring next sample up to volume) (Rinse beakers and flasks in between samples with warm tap water and DI water, dry out beakers before next sample is added)
- 15.) After sample is mixed, gravity filter sample into a 15 mL glass culture tube using Whatman 541 filter paper (hardened, ashless, fast filter speed). Filtrate can be stored for 2 weeks at 4°C until analysis of hydroxyproline, but analyze as quickly as possible (Plan for same day analysis).

C. Hydroxyproline Assay

Limit the number of tubes per assay to 150 or less because of the time constraints in pipetting steps and in reading on the spectrophotometer. Also, make sure you have enough chemicals, supplies, and reagents to read all the samples for the group. Pre-label the culture tubes to save time prior to reading.

Reagents:

Make the first three solutions prior to beginning hydroxyproline assay

600 µg/mL Stock Hydroxyproline Standard:

In a 50-mL volumetric flask, dissolve 30 mg hydroxyproline in MQ H₂O. Mix thoroughly and transfer to a 50-mL plastic conical tube, and store at 4°C for up to 2 months. (Make sure to get all of hydroxyproline into flask, weigh out hydroxyproline in flask to alleviate any problems with transferring).

Buffer solution:

- 1.) In a 1-L glass beaker filled with 500 mL of MQ H₂O, dissolve while stirring:
 - 30g Citric acid monohydrate
 - 15g Sodium hydroxide
 - 90g Sodium acetate trihydrate
- 2.) Add 290 mL 1-propanol. Mix vigorously. At this point, if this solution is not mixed continually, it will separate into layers.
- 3.) Adjust the pH to 6.0 with concentrated HCL
- 4.) Transfer to 1-L volumetric flask and bring up to volume using MQ H₂O. Store in a labeled, glass bottle covered in foil at 4°C for up to 1 month. Before using, make sure solution has not separated into layers again.

60% Perchloric acid: *Work in a fume hood and wear safety glasses*

- 1.) In a graduated cylinder measure out 85.7 mL of 70% perchloric acid.
- 2.) Bring up to 100 mL with MΩ H₂O.
- 3.) Transfer to labeled, 100-mL glass bottle. Store at 4°C for up to 1 month.

Make the following solutions same day, and just before adding the solution to the first set of tubes.

Chloramine-T Oxidant Reagent: *Wear a mask when weighing out the Chloramine-T*

- 1.) Dissolve 1.41g chloramine-T in 100 mL of Buffer solution.
- 2.) Make fresh daily, approx. 20 mL over what is needed to pipette 1 mL into each tube (Ex. 3 samples × 2 hydrolysates × 2 duplicates + 16 std. curve = 28, make 50 mL of solution.)

DMBA (dimethylaminobenzaldehyde) Color Reagent:

- 1.) In a 100 mL beaker, dissolve 10 g of 4-dimethylaminobenzaldehyde in 35 mL of cold 60% perchloric acid.
- 2.) Slowly add, with stirring, 65 mL of 2-propanol (isopropyl alcohol)

Protocol:

- 1.) Set water bath to 60°C and preheat prior to reading (Takes approx. 30-45min to get to correct temperature)
- 2.) Label culture tubes the previous day to save time or before beginning the color assay
- 3.) Prepare the 6 μg/mL Working Hydroxyproline Standard: pipet 1 mL of 600 μg/mL Stock Hydroxyproline into a 100-mL volumetric flask. Bring up to volume with MΩ H₂O. Mix thoroughly.
- 4.) Using the repeater pipet, pipet 1.50 mL of MΩ H₂O into all insoluble culture tubes and 1.0 mL of MΩ H₂O into all soluble tubes.

5.) Prepare the standard curve using the following table:

Table D.1 Hydroxyproline standard curve volumes.

Standard Number	Volume of 6 $\mu\text{g/mL}$ Working Hydroxyproline standard, mL	Volume of $\text{M}\Omega \text{H}_2\text{O}$, mL	Final Volume, mL	Hydroxyproline Final Concentration, $\mu\text{g/mL}$
Blank	0.000	2.000	2.000	0.000
S-1	0.025	1.975	2.000	0.075
S-2	0.050	1.950	2.000	0.150
S-3	0.100	1.900	2.000	0.300
S-4	0.200	1.800	2.000	0.600
S-5	0.400	1.600	2.000	1.200
S-6	0.600	1.400	2.000	1.800
S-7	0.800	1.200	2.000	2.400

a. Order of the tubes for the standard curve is as follows:

Blank, S-1, S-2, S-3, S-4, S-5, S-6, S-7

6.) Using a repeater pipet with 50-mL combi-tip attached, add 1.0 mL of Chloramine-T

Oxidant Reagent to all standard curve tubes. Vortex to mix (set vortex to 7 or less.) Let stand at room temperature for 20 ± 2 minutes.

7.) While incubating the standard curve, begin pipetting samples into culture tubes (For insoluble: 1.50 mL $\text{M}\Omega \text{H}_2\text{O}$, 0.5 mL of sample; for soluble: 1.0 mL $\text{M}\Omega \text{H}_2\text{O}$, 1.0 mL of sample. *(Limit the group amount to 12 samples and space incubation periods approx. 10 mins apart from one another.)*)

8.) During incubation of Chloramine-T, prepare sufficient amounts of DMBA Color Reagent to complete the assay

9.) After incubation of chloramine-T, add 1.0 mL of DMBA Color Reagent using a repeater pipet to tubes. Vortex to mix, cover with aluminum foil, and incubate in a water bath set to 60°C for 15 minutes (timing is critical).

10.) After incubation in water bath is complete, remove tubes and move them to a cold tap water bath for at least 3 mins

- 11.) Pipet 1 mL of sample to cuvette and read absorbance of samples against the water BLANK on a UV/Vis Spectrophotometer set to 558 nm. Reading should be completed within 1 hr.
- 12.) Using the standard curve absorbances and known concentrations, the GEN5 software will generate a linear regression equation and calculate the initial concentration of the unknown samples.
- a. Check the standard curve R^2 should be 0.995 to 1.0. If not, delete/mask the bad points.
 - b. Are the slope and intercept similar to previous hydroxyproline assays?
 - c. Is the control within control limits?
 - d. Do the unknown sample values fall within the standard curve?
 - e. Are the sample values in normal range or what you expected?
 - f. Are the CV of the unknown samples $\leq 5\%$? If not, consider repeating the assay on new duplicate dilutions of the unknown samples.