DISSERTATION

BEEF TENDERNESS AND THE MANAGEMENT OF CALF-FED HOLSTEIN STEERS TO MEET MARKET STANDARDS

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2013

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ABSTRACT

BEEF TENDERNESS AND THE MANAGEMENT OF CALF-FED HOLSTEIN STEERS TO MEET MARKET STANDARDS

Tenderness is one of the most influential sensory attributes determining consumer acceptance of beef products. Beef at retail represents production of a diverse cattle population, including both beef breeds and cattle bred for milk production. Objectives of this work were to first benchmark tenderness at the retail level and then determine appropriate management strategies to maximize quality and yield of calf-fed Holstein steers. Fifty-four stores in thirty U.S. cities were sampled from June 2011 through May 2012 to benchmark tenderness of beef steaks at retail as assessed by Warner-Bratzler shear force (WBSF). Top loin (N = 980) and sirloin (N = 860) steaks were purchased and shipped via overnight delivery to Colorado State University, Fort Collins, CO. The survey was divided into two periods based on samples shipped fresh and frozen on arrival (Period 1) or samples shipped frozen and stored frozen (Period 2). Mean WBSF values during Period 1 were 2.9 and 3.9 kg for top loin and sirloin steaks, respectively. Frequencies of steaks classified as tough (WBSF $\geq$ 4.4 kg) were 8.6% and 17.7% for top loin and sirloin steaks, respectively. Examination of coefficients of variation associated with means reflecting the influence of freezing, retail display and shipping suggested that variance remained unchanged ($\pm$ 2.0%) with respect to shear force values; however, mean shear force values were reduced as a result of shipping conditions. Mean WBSF values during Period 2 were 3.4 and 4.0 kg for top loin and sirloin samples, respectively. Frequencies of steaks classified as tough were 14.3% and 24.8% for top loin and sirloin steaks, respectively.

Calf-fed dairy steers comprise approximately 10% of fed-beef harvested in the United States, annually (Moore et al., 2012). This population of cattle is much different genetically and requires use of growth promotants to meet comparable feedlot performance to that of beef breeds. The effect of beta-agonist supplementation on live performance, carcass characteristics, fabrication yields and beef quality of calf-fed Holstein steers was investigated using steers implanted with a combination trenbolone acetate/estradiol based implant and blocked by initial weight into pens (N = 32). Pens consisted of 90 steers each and were randomly assigned to one of four management strategies including: implant only, ractopamine hydrochloride (RH) fed at 300 mg/hd/d for the final 30 d of finishing or RH fed at 400 mg/hd/d for the final 30 d of finishing, and zilpaterol hydrochloride fed at 6.8 g/ton for 23 d with a 3 d withdrawal prior to harvest. Feed efficiency was improved in beta-agonist fed steers 18 to 25% and hot carcass weight was increased by 1.8 to 3.7% ($P < 0.05$). Beta-agonists increased saleable yield by 0.6 to 1.9%, decreased fat by 0.6 to 1.3% and shifted tissue distribution such that a greater percentage of side weight was comprised of the muscles of the round ($P < 0.05$). Changes in development were observed as a result of beta-agonist use, specifically as an increased proportion of weight comprised of muscles of the hindquarter ($P < 0.05$). Use of beta-agonists negatively impacted shear force and sensory attributes. Beta-agonists had no effect on marbling; however, supplementation using any treatment increased shear force by 9 to 26%. Zilpaterol hydrochloride reduced trained panel ratings for tenderness, juiciness and flavor, but this was not observed in beef from steers treated with RH at 300 mg/hd/d. These effects were nearly linear as dose and potency of beta-agonists increased. The most aggressive beta-agonist treatments increased incidence of samples failing to be certified as tender from just over 10% in controls to approximately 20 to 25% at 21 d postmortem ($P < 0.05$). To produce beef comparable to current

tenderness levels at retail, producers must appropriately manage use of beta-agonists and implants in populations of calf-fed Holstein steers.

ACKNOWLEDGEMENTS

The successes I may have had throughout my personal and professional life are a result of the amazing group of people who surround me. My mother and father instilled in me a love for agriculture and learning, while teaching me that no amount of scholastic accomplishment will surpass the value of work ethic. My sister has challenged me intellectually, allowing me the opportunity to grow beyond my own school of thought. My closest friends, Brandon McEndaffer, Michael Rea, Michael Macklin, Michael Harvey, Dustin Rippe, Ryan Hodack, Colin Durham and Nate Moll have grown into a network of professionals that are second to none. This group constantly reminds me the best ideas are realized during good times with great people.

The list of professional mentors I have had begins again with my parents. The knowledge they share never ceases to amaze me and both of them constantly remind me of the class that is required to be a professional. Dr. Keith Belk has challenged me to grow as a scientist and a person. He has taught me how to think, how to question and how to evaluate – lessons that will apply universally. Dr. Dale Woerner has taught me professionalism, work ethic and leadership, as well as reminding me of the character it takes to succeed in this world. The talks I have shared with Dr. J. Daryl Tatum have been some of my fondest memories at Colorado State. He is the best educator I have encountered and the knowledge he can impart in a five minute conversation is second to none. Dr. John Scanga first exposed me to advanced meat science, scientific writing and professional presentations, in doing so he served as a tremendous advisor through my years as an undergraduate and graduate student. Dr. Mo Salman was a fantastic committee member, expanding my horizons well beyond the typical realm of meat science research. Dr. Derek Vote gave me exposure to the industry that I value as much as any

classroom experience. My judging coaches, Gail Goehring, Todd Taylor, Travis Hoffman, Chancie Moore and Shane Bedwell are second to none; no amount of writing can explain what judging has done for my development as a person and a professional.

To my colleagues – Drs. Jessica Igo and Rebecca Acheson, words cannot express the thanks I owe to both of you. You are both simply amazing people. Drs. Travis O'Quinn and Travis Arp, your help was second only to the fun I had with our day-to-day conversations. Dani Shubert, Dan Sewald, Erin Karney, Jordan McHenry, Carlie Perham, Curtis Pittman, Santiago Luzardo, Xiang Yang, Jessica Steger, Lenise Mueller and Megan Webb – your selflessness in completing the projects contained in this work was amazing. Finally, I must thank a group of people who I was charged with educating, but who taught me more than I could have ever dreamed. To the members of the judging and quiz bowl teams I was blessed with coaching – watching you grow into professionals is the greatest joy I have had during my tenure at Colorado State University. I hope I taught you even half as much as you taught me.

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CHAPTER I

INTRODUCTION

Beef is a $79 billion industry that attempts to provide consumers with a uniform, high quality product. These products are part of over 25 billion pounds produced annually from a cowherd of just over 90 million head (USDA-ERS, 2013). The cowherd in the U.S. is an exceptionally diverse population, including animals bred specifically for either meat or milk production. Both segments eventually contribute to the fed-beef supply and influence consumer demand. In 2011, dairy-type carcasses comprised nearly 10% of fed-beef presented for grading in commercial facilities (Moore et al., 2012). The packing sector is the intersection of beef breeds and dairy type cattle for purposes of beef production. Variation in selection criteria between, and within these populations result in extreme genetic diversity within the U.S. fed beef industry. The influence of genetics on beef quality may not be totally understood, but it is unquestionable that genetics impact both marbling and tenderness (Tatum, 2006).

Beef tenderness has been one of the most thoroughly investigated topics in the field of meat science. Summaries exist that have addressed pre-harvest influences (Tatum, 2006; Tatum et al., 2007), post-harvest interventions (Smith et al., 2008) and prediction of beef tenderness (Woerner and Belk, 2008). Monitoring of beef tenderness has been achieved through national surveys of retail locations (Morgan et al., 1991; George et al., 1999; Brooks et al., 2000; Voges et al., 2007; Savell, 2012). These works have demonstrated a trend for improved beef tenderness over the time (Table 1.1). Despite this progress, the beef industry faces declining consumer demand (LMIC, 2013) in a market place that has demonstrated increased willingness-to-pay for more tender, or guaranteed tender products (Boleman et al., 1997; Platter et al., 2005). This

contrast necessitates constant monitoring of tenderness at the retail level, as well as evaluation of the mechanisms used to assess the trait.

Production of tender beef products must be addressed from the perspective of total-quality management with consideration of genetics, nutrition, growth promotants, animal health, animal handling and postmortem management of rigor. Cattle producers are forced to balance good management practices as they relate to product quality, with practices to improve efficiency that are known to be deleterious to sensory attributes (Dikeman, 2007). The U.S. beef industry is reliant on ionophores and growth promotants such as hormone based implants and beta-agonists to maintain profitability or minimize economic losses. Estimates in 2005 suggested that prices would need to increase 36% if growth promoting technologies were removed from beef production (Lawrence and Ibarburu, 2007). The economic impact of growth promotants may be even greater in populations of cattle that require additional days on feed to reach market weight, such as calf-fed Holstein steers.

Calf-fed Holstein steers are raised in a manner such that feed resources comprise a greater portion of the total cost of production compared to beef breeds. Beef breeds typically begin life on pastures, nursing their dam for approximately the first six months. This is routinely followed by additional time on grass in the stocker sector of the beef industry, before placement into feedlots for an approximately 100 to 150 d finishing period. In contrast, calf-fed Holstein steers will spend only hours with their dam, being placed almost immediately on supplemental milk rations. The majority of dairy calves spend the first months of life on calf ranches that specialize in growing these animals to approximately 300 lbs (Duff and Anderson, 2007). Calves will then enter feedyards and possibly spend over 300 days on feed to reach market weight (Duff and McMurphy, 2007). Although dairy type feeder cattle are significantly cheaper

to purchase, the feed resources required to reach market weight may outweigh the initial price advantage. Additionally, dairy type cattle have been reported to consume more (Fox et al., 1988) and be less efficient compared to beef breeds (Duff and McMurphy, 2007).

Growth promoting technologies offer an option to improve efficiency and yield of calf-fed Holstein steers. Hormone based implants have been investigated as growth promotants in ruminants since the 1940's (Raun and Preston, 1997). These compounds have been found to increase DMI and ADG in proportions that improve efficiency (Perry et al., 1991; Apple et al., 1991; Duckett et al., 1997). Over 98% of cattle receive at least one implant, of which 80% receive two or more (NAHMS, 2000). Most calf-fed Holstein steers will receive an implant upon arrival at the feedyard and then be re-implanted with a terminal implant before harvest. Implants used in successive phases of beef production are estimated to add over 45 kg (Duckett and Andrae, 2001). More recently, the additive effect of beta-agonists has been explored in both beef breeds and calf-fed Holstein populations. Two commercially available beta-agonists are approved for use in cattle in the U.S. Ractopamine hydrochloride (Optaflexx®, Elanco Animal Health, Greenfield, IN), was approved by the FDA for use in cattle in 2003, followed by zilpaterol hydrochloride (Zilmax®, Merck Animal Health, Summit, NJ) in 2006. Fed for the last 20 to 42 days of the finishing period, these compounds increase efficiency, dressing percentage, muscle to bone ratio and subprimal yield, while decreasing fat (Avendaño-Reyes et al., 2006; Scramlin et al., 2010; Arp, 2012).

The advantages in live performance and yield following use of implants and beta-agonists are contrasted by the negative effects these products have on meat quality. Tatum (2006) summarized the effect of several types of implants, used singly or in combination. This work reported that more aggressive androgenic implants, as well as use of multiple implants, resulted

in reduced tenderness as assessed by shear force determination (Tatum, 2006). Beta-agonists have also been reported to increase shear force values. The effect of ractopamine hydrochloride (RH) on beef quality has been reported to be milder than that of zilpaterol hydrochloride (ZH); however, both compounds negatively impact tenderness (Avendaño-Reyes et al., 2006; Scramlin et al., 2010; Gruber et al., 2008; Arp, 2012). Reduced marbling has been documented to coincide with reduced tenderness in cattle fed beta-agonists (Avendaño-Reyes et al., 2006; Scramlin et al., 2010; Arp, 2012).

Calf-fed Holstein steers have been found to produce carcasses that have high levels of marbling (Garcia de Siles et al., 1977; Nour et al., 1981; Nour et al., 1983; Thonney et al., 1984; Knapp et al., 1989; Perry et al., 1991). The inherently high levels of marbling in calf-fed Holstein steers is likely due to genetic potential, early weaning, high plane of nutrition and extended days on feed (Zinn et al., 1970; Myers et al., 1999; Myers et al., 1999b; Shike et al., 2007). These factors could act to negate some of the deleterious effects of growth promotants when used in this population. The effects of implants and beta-agonists have been explored in calf fed Holstein steers, with similar conclusions to those found within populations of beef breeds (Apple et al, 1991; Perry et al., 1991; Bass et al., 2009; Vogel et al., 2009; Beckett et al., 2009). Unfortunately, no work to date has evaluated the effect of using both RH or ZH in a contemporary sample population of calf-fed Holstein steers.

Table 1.1. Summary of sample population means for Warner-Bratzler shear force (WBSF) of top loin and sirloin samples collected at retail during major tenderness surveys.

	Top Loin Steak WBSF (kg)		Sirloin Steak WBSF (kg)	
	Mean	≥ 3.9[a] (%)	Mean	≥ 3.9[a] (%)
Morgan et al., 1991	3.25	4.0 – 21.0[b]	3.56	4.0 – 21.0[b]
George et al., 1999	1.91 – 3.19[b]	13.3	2.72 – 3.54[b]	20.5
Brooks et al., 2000	2.77	6.6	3.04	11.0
Voges et al., 2007	2.12	0.0	2.50	0.0
Savell, 2012	2.36	4.3	2.45	2.2

[a] WBSF $\geq$ 3.9 indicates samples predicted to be intermediate or tough in terms of tenderness (Platter et al., 2005).

[b] Data separated by quality grade. Range represents inclusion of all grades analyzed.

CHAPTER II

REVIEW OF LITERATURE

Calf-Fed Holstein Steers

Calf-fed Holstein steers represent a consistent, high quality supply of beef. National statistics approximate that there are 9.2 million dairy cows in the U.S, a number that has remained relatively constant even while the population of beef cows has declined drastically (NASS, 2012). Annually, the dairy cow portion of the nation's herd has been estimated to produce between 2.4 and 3.0 million bull calves (Shaefer, 2005; Cheatham and Duff, 2004), accounting for nearly 10% of fed-beef harvest (Moore et al., 2012). This number was little changed from an estimate by Wellington (1970) from Henderson (1969) who calculated that 12% of cattle on feed were of dairy lineage. This population descends from an exceptionally homogeneous gene pool as a result of single trait selection for milk production (Shaefer, 2005). This limited genetic base yields a consistency in type and kind not attainable through harvest of beef breeds. Consequently, producers are more accurately able to target the strengths and manage the weaknesses of calf-fed Holstein steers.

Carcass Performance

Management of calf-fed Holstein steers is substantially different than management of beef breeds. Most calf-fed Holsteins enter a feedyard at approximately 300 pounds, spend over 300 days on feed, and exit at weights between 1300 and 1400 pounds (Rust and Abney, 2005). Increased days on feed and early weaning have been found to increase marbling at time of harvest (Zinn et al., 1970; Myers et al., 1999; Myers et al., 1999b; Shike et al., 2007). High levels of marbling and low levels of external fat have been noted in calf-fed Holstein steers

(Wellington, 1973; Garcia de Siles et al., 1977; Nour et al., 1981; Nour et al., 1983; Thonney et al., 1984; Knapp et al., 1989; Perry et al., 1991; Abney, 2004; McKenna et al., 2002; Garcia et al., 2008; Moore et al., 2012). McKenna et al. (2002) and Garcia et al. (2008) provided evidence of higher quality grades in dairy type carcasses compared to carcasses from beef breeds. Garcia et al. (2008) and Moore et al. (2012) reported reduced physiological maturity in dairy type carcasses. Reduced physiological maturity could result in more tender beef products; however, the difference in age between calf-fed Holstein steers and animals from beef breeds is likely not significant enough for age alone to account for observed differences in tenderness.

Studies that have compared beef products from beef breeds to those from calf-fed dairy steers have cited greater tenderness of beef from calf-fed dairy steers (Knapp et al., 1989; Thonney et al., 1991). These findings have been contrasted by other works which have failed to find a difference in tenderness when beef from calf-fed Holstein steers was compared to that from beef breeds (Ramsey et al., 1963; Armbruster et al., 1983; Shaefer et al., 1986). Reasons for these different results could relate to variations in breed, type and kind of beef cattle being compared to the calf-fed dairy population. In either instance, all of the previously cited works have demonstrated that beef from calf-fed Holstein steers is comparable to, if not superior in terms of sensory attributes, to products from beef breeds.

Additional value from calf-fed Holstein carcasses may be found in differences in trim items and by-products. Due to reduced external fat, trim from calf-fed Holstein steers is typically higher in lean content and may be rewarded a premium (Siemens, 1996). Schaefer (2005) summarized the work of Buege in who stated that hides from calf-fed Holstein steers may also be more valuable as they are larger, thinner and typically non-branded; a value reducing practice common among beef breeds. Nevertheless, despite advantages in quality, reduced

external fat and increased value of certain by-products, calf-fed dairy steers are typically discounted by packers.

Calf-fed Holstein steers have been reported to have low dressing percentage due to reduced fat and conformation scores (Knapp et al., 1989; Perry et al., 1991), coupled with increased size of both the gut and liver (Taylor and Murray, 1991). Most frequently, it has been cited that calf-fed Holstein steers have smaller ribeye areas compared to beef breeds (REA) (Wellington, 1971; Bertrand et al., 1983; Knapp et al., 1989; Perry et al., 1991). Also, calf-fed dairy steers have been documented to have substantially greater amounts of kidney, pelvic and heart fat (KPH) (McKenna et al., 2002; Moore et al., 2012). However, Nour et al. (1983) showed that KPH may be comparable at lower live weights when comparing beef breeds and calf-fed dairy type animals. The 2005 National Beef Quality Audit found no difference in KPH measurements dairy type carcasses compared to carcasses from beef breeds (Garcia et al., 2008). The three most recent National Beef Quality Audits have presented contrasting evidence related to differences in hot carcass weight (HCW) in dairy type carcasses (Mckenna et al., 2002, Garcia et al., 2008; Moore et al., 2012). All of these works showed HCW of dairy type cattle to be either comparable to or greater than carcasses from beef breeds. The combined possibility for increased HCW and KPH with reduced REA could increase numeric USDA yield grade. However, only the work of McKenna et al. (2002) made such a conclusion.

Cutability of calf-fed Holstein steers may or may not be reflected accurately by USDA yield grade (Lawrence et al., 2010). The USDA yield grade equation fails to consider muscle to bone ratio, which is lower in calf-fed dairy cattle compared to beef breeds (Knapp et al., 1989). The work of Knapp et al. (1989) explained that the cutability advantage gained in calf-fed Holsteins due to reduced fat may be lost when percent bone is considered. Moreover, Nour et al.

(1981) reported that muscle to bone ratio also is lower in Holstein versus Angus steers. Nevertheless, the work of Thonney et al. (1984) found that as carcass weight increased, cutability of calf-fed Holstein steers was less negatively impacted compared to Angus cattle. Previous work explained this phenomenon on the premise that at similar weights, calf-fed Holstein steers will deposit less intermuscular (seam) fat in both the rib and chuck (Thonney et al., 1984). This conclusion was subsequently substantiated by the work of Knapp et al. (1989) who reported that, when subprimals were fabricated into cuts with less external fat, trimmer cattle generated greater carcass yields. Knapp et al. (1989) determined that as external fat levels were trimmed from 2.54 cm to either 0.0 or 0.64 cm, cutability advantages emerged in favor of calf-fed dairy cattle compared to beef breeds. Nour et al. (1983b) found an increased subprimal yield in carcasses from calf-fed Holstein steers compared to beef breeds.

Advantages that carcasses from calf-fed Holstein cattle possess in subprimal cutability led Shaefer (2005) to conclude that dressing percentage was the major reason for packer discounts. However, other workers have cited that cut size and shape may also be a concern at the packing and retail level (Thonney et al., 1991). These issues were most apparent when considering discounts and premiums paid to packers for subprimals originating from calf-fed Holstein carcasses. In today's market, subprimal cuts derived from the chuck and round of calf-fed Holsteins typically receive a premium, whereas cuts from the rib and loin are discounted. Premiums are based on higher retail yields in subprimals from the chuck and round, whereas discounts are applied to middle meats due to the small and narrow shape of the *Longissimus dorsi* (Lawrence et al., 2011). A final financial consideration for packers who purchase calf-fed Holstein steers is that this population is more likely to experience increased incidence of liver condemnation due to abscess resulting from increased days on feed (Duff and McMurphy, 2007).

Feedlot Performance

Holstein feeder calves enter finishing operations at a substantially different physiologic status compared to their beef contemporaries. Aside from differences in weight, management of Holstein feeder calves during the first months of life is such that they are exposed to a wider range of stressors compared to beef breeds (Cheatham and Duff, 2004). Duff and Galyean (2007) cited a number of factors influencing immunity which include pre-weaning considerations such as vaccination and colostrum intake, as well as post-weaning factors like transportation, co-mingling and nutrition. It should be noted that pre-weaning vaccination with live virus and modified live viruses may have varying levels of efficacy based on passive immunity acquired from colostrum. The structure of the dairy industry is such that bull calves, typically only a few days old, are shipped to calf ranches for development before entering the veal or beef supply chain (Duff and Anderson, 2007). Extensive preventative health measures are utilized upon arrival at the calf ranch, after which calves are trained to eat at bunks and exposed to concentrate rations (Duff and Anderson, 2007). This system of development places calf-fed Holstein steers entering feedlots at a possible advantage in terms of immunity, stress tolerance and adaptation to feeding practices compared to beef breeds received off pastures.

Finishing of calf-fed Holstein steers requires a substantially greater number of days on feed (Duff and McMurphy, 2007). The added time required to reach a logical market endpoint reduces the throughput of cattle in the finishing operation. Based on a standard finishing period of 3 to 6 months for beef breeds, a typical feedyard is capable of 2 to 2.5 rotations of its capacity each year. If calf-fed Holstein steers require upwards of 300 days on feed, the same yard would be expected to make slightly over one rotation of its capacity each year. Depending on supply of feeder calves, this could be advantageous as risk management practices can be used much more

precisely based on a constant supply, relatively constant cost for feeder calves and an easy calculation of required feed supply.

Regarding performance of calf-fed Holstein steers relative to beef breeds, consumption and efficiency are of importance. During the finishing phase, calf-fed Holsteins have been reported to consume roughly 8% more on a DM basis (Fox et al., 1988). Previous works reached contrasting conclusions when the growth of calf-fed Holstein steers was compared to beef breeds. Several works found improved growth rates and efficiencies in Holstein cattle compared to British breeds (Garcia-de-Siles et al., 1977; Thonney, 1987). These workers suggested that improved efficiency at comparable weights ensued due to increased frame size of the calf-fed Holstein cattle. These findings were contrasted by those of Gareett (1971) who found improved efficiency in beef breeds. Selection of beef breeds for improved growth and frame has likely negated the performance advantages initially reported in Holstein steers. This is reflected in the work of Perry et al. (1991) who found improved efficiency and ADG in both Angus and Angus x Simmental steers relative to calf-fed Holsteins. A summary of data presented by Duff and McMurphy (2007) confirmed that beef breeds are more efficient and have higher average daily gains than Holsteins.

The need to improve efficiency, average daily gain, dressing percentage and muscle to bone ratio encourages adoption of pre-harvest management strategies that use growth promotants. These strategies could include use of hormone based implants or beta-agonists synergistically with ionophores. Ionophores and hormone based implants have been extensively studied in feedlot cattle. The rest of this review will focus on the mechanisms, effects and cost-benefit analysis of using ionophores and growth promotants in calf-fed Holstein steers.

Ionophores

Carboxylic polyether ionophore antibiotics are produced by *Streptomyces* and were originally developed for use as an anticoccidial feed additive in poultry production systems (Bergen and Bates, 1984). These compounds have been approved for use in ruminant diets since the mid-1970's (Russell and Strobel, 1989). Monensin (Rumensin®), lasalocid (Bovatec®), salinomycin, narasin and laidlomycin propionate (Cattlyst®) are all examples of ionophores (Bergen and Bates, 1984). Russell and Strobel (1989) estimated that at a feed efficiency of 8 to 1 (pound of feed to pounds of gain), the value of ionophores to the beef industry in terms of feed cost savings was over $500 million, a number that may have increased with increased feed prices, or declined due to improved efficiency as a result of other growth promotants.

Chen and Wolin (1979) showed that ionophores inhibit growth of gram positive bacteria in the rumen. Gram positive bacteria are primarily responsible for production of lactate, the compound associated with sub-acute acidosis (Slyter, 1976; Bergen and Bates, 1984). Selection for gram negative bacteria favors the production of succinate, the precursor to the volatile fatty acid propionate. Propionate is more efficiently utilized by the animal due to increased enthalpy and oxidation potential (Russell and Strobel, 1989). Ionophores have been reported to decrease methane production by approximately 30% (Schelling, 1984) resulting in greater carbon and energy retention by rumen (Richardson et al., 1976). Additionally, ionophores decrease protein degradation to ammonia and volatile fatty acids, increasing rumen by-pass proteins for metabolic functions (Dinius et al., 1976). The result is a decline in feed intake, no impact on daily gain, and an increase in feed efficiency (Bergen and Bates, 1984).

Ionophores rely on exchange of protons and cations across the cell membrane to function. Particular ionophores have greater affinities for different cations, Na^+ in the case of Monsensin

and K^+ in the case of lasalocid. The influx of protons into the cell dissipates the proton motive force necessary to generate ATP (Bergen and Bates, 1984). Bergen and Bates (1984) summarized the work of Rosen and Kasket (1978) who found that anaerobic bacteria are dependent on hydrogen entering the cell to drive cellular ATPase that re-phosphorylates ADP. Lipophilic ionophores have been shown to form a pore in the cellular membrane allowing protons to enter via pathways other than those responsible for ATP generation. The entry of protons occurs in exchange for passage of cations outside the cell (Russell, 1987). Eventually, proton motive force is eliminated as no hydrogen ions are able to enter, thus no ATP is generated and the cell lyses. Conveniently, the gram negative bacteria favored by ionophores are capable of cellular respiration. In the presence of ionophores, production of succinate occurs through an oxidation-reduction reaction that generates proton motive force for purposes of ATP production (Bergen and Bates, 1984). Ultimately, succinate is converted to propionate.

In a meta-analysis of over 64 works that evaluated the effect of ionophores on feedlot performance, Duffield et al. (2012) found that monensin increased feed efficiency 6.4%, and ADG 2.5%, coupled with a 3% decrease in DMI. Ionophores also offer advantages for addressing challenges specific to the calf-fed Holstein steer population. Vogel and Parrott (1994) reported an increased mortality rate in calf-fed Holstein steers compared to beef breeds. The leading cause of death in calf-fed Holstein steers was digestive issues, which contrasted the population of beef breeds in which respiratory disease was the number one cause of death (Vogel and Parrott, 1994). Digestive issues (bloat, acute and sub-acute acidosis) in calf-fed Holstein steers likely arise as a result of extended days on feed (Smith, 1998). Increased days on feed coupled with increased digestive upset and DMI makes calf-fed Holstein steers more prone to liver abscess (Nagaraja et al., 1996; Nagaraja and Chengappa, 1998). The most recent National

Beef Quality Audit cited an incidence of liver condemnation of approximately 20%, over half of which occurred due to major or minor abscess (McKeith et al., 2012). Smith (1998) estimated that liver condemnations cost the beef industry approximately $36 million per year. Export liver value is approximately $0.65/pound compared to $0.10/pound in the rendering industry (Erin Borror, personnel communication, June 26, 2013). Packers estimate that each liver has an approximate average weight of 15 pounds. Incidence of liver abscesses in calf-fed Holstein steers recently spiked to 20 to 40%, with some observations in excess of 50%. With an annual fed beef harvest of 26 million head (USDA-ERS, 2013), 10% of which is comprised of dairy-type steers (Moore et al., 2012); liver abscess in Holstein steers costs the industry between $4.3 million and $8.6 million/year. This figure also does not account for potential trim losses due to liver abscess.

Fewer studies have investigated the effect of ionophores on calf-fed Holstein steers than in beef breeds of cattle. Initial research evaluated the effect of monensin on Holstein steers and showed increased concentration of propionate in the rumen, reduced feed intake and increased feed efficiency; however, these trials all involved limited sample sizes of 46 to 80 steers (McKnight et al., 1980). In later work, Ramirez et al. (1998) investigated the effect of laidlomycin propionate (LP) on calf fed Holstein steers. Supplementation with LP increased ADG by 6.3 to 9.7% and feed efficiency by 4.2 to 4.5%. Other work using cannulated Holstein steers reported that LP decreased rumen degradation of N (protein) by over 10% and increased N digestibility and microbial efficiency (Zinn et al., 1996). In a separate trial, LP reduced rumen pH in four cannulated Holstein steers (Zinn et al., 2000). Effects of monensin on calf-fed Holstein steers were explored by Lana et al. (1997) who found a 1 to 3% increase in ADG and feed efficiency at two differing levels of monensin, fed with two different nitrogen sources. The

same work showed significant increase in gain per unit of crude protein intake when monensin was increased from 0 to 33 mg/kg (Lana et al., 1997). Monensin also was reported to decrease incidence of bloat in cannulated Holstein steers by approximately 35% as monensin levels were increased from 0 to 40 g/ton (Coe et al., 1996). Similar work comparing effects of monensin fed at 30 or 40 g/ton to approximately 1,000 calf-fed Holstein steers showed a strong trend (P = 0.06) for reduced digestive mortality in calves fed 40 g/ton (Laudert et al, 1994). Reduced mortality, digestive upset and improved efficiency make iononphores a practical option for feedlot producers that raise calf-fed Holstein steers. Interestingly, the relative improvement in feed efficiency through use of monensin has decreased over the past four decades (Duffield et al., 2012). This could be due to improvements in genetics, use of other growth promotants such as implants and beta-agonists, or development of resistance to ionophores.

Hormone-Based Implants - History

The first work examining use of hormones for purposes of growth promotion in ruminants was conducted at Purdue University in 1947 (Raun and Preston, 1997). This work evaluated the effect on performance of the estrogenic compound diethylstilbesterol (DES) and testosterone injected into spayed Hereford heifers and found increased gain and efficiency could be achieved through administration of DES (Dinusson et al., 1948). Hale (1953) first investigated oral administration of DES to ruminants, which led to the work of Burroughs (1954) who reported a 35% increase in gain coupled with a 20% reduction in feed cost. This technology was quickly patented by Iowa State College and licensed by Eli Lilly and Co., Inc. (Raun and Preston, 1997).

The FDA approved DES for use in beef cattle in 1954 (Raun and Preston, 1997). During the peak of DES use by livestock producers, it was estimated that 80 to 95% of fat cattle received

the compound (Raun and Preston, 1997). By 1979, following negative press and a study that demonstrated the carcinogenic nature of DES when a massive dose was administered to pregnant women, FDA was forced to ban the substance for use in cattle (Raun and Preston, 1997). However, this process paved the way for approval of zeranol (1969), silastic estradiol (1982), trenbolone acetate (TBA) (1987) and combination TBA-estradiol based implants. Recent estimates have reported over 98% of feedlot cattle receive one or more implant and nearly 80% receive two or more (NAHMS, 2000).

Hormone-Based Implants – General Effects

Research on the effect of administering hormones to livestock species demonstrated potential for increased growth (Dinusson et al., 1948; Hale et al., 1953; Burroughs et al., 1954). Continued exploration of these topics revealed that the compounds also increased DMI (Clegg and Cole, 1954; Klosterman et al., 1955; Deans et al., 1956; Forrest and Sather 1965); however, increased feed consumption was out-paced by gain, resulting in improved efficiency (Wilkinson et al., 1955; Burgess and Lamming, 1960; Wallentine et al., 1961). Compositional differences were noted by Gee and Preston (1957) who found improved protein deposition in cattle treated with implants. Preston (1975) hypothesized that this occurred in conjunction with reduced fat in the carcass. These observations have been substantiated by work over the past half-century.

Despite clear evidence supporting the positive effect of hormone supplementation on growth, early work led to significant debate as to the mode of action by which estrogens elicited these responses. Preston (1975) summarized that the general metabolic response to estrogen included an increase in the size of the anterior pituitary, accompanied by increased levels of growth hormone (GH). Additionally, it was noted that estrogen caused increased retention of nitrogen, calcium and phosphorus (Preston, 1975). Preston (1975) reported five potential modes

of action, which included increased production of adrenocorticotropic hormone (ACTH), growth hormone (GH), insulin and thyroid hormone, or a direct effect of estrogens on tissues.

Hormone-Based Implants – Mode of Action

Further research has helped to demonstrate the real mode of action for estrogens and androgens may involve some combination of those hypotheses proposed by Preston (1975). Early observations that pituitary weight was increased with administration of GH were substantiated by Trenkle (1997), who summarized that increased numbers of GH-secreting cells followed administration of trenbolone acetate (TBA) and estradiol. Increased levels of circulating GH was not reported in steers implanted with TBA alone (Hayden et al., 1992). Further work investigating the role of GH in mediating the response to administration of estrogens examined increased sensitivity of steers to GH-releasing hormone (GHRH). The hypothalamus serves as the source for GHRH, which causes the anterior pituitary to release GH (Trenkle, 1997). Hongerholt et al. (1992) reported that administration of estrogens coupled with GHRH resulted in increased amounts of GH to be released. This led the authors to conclude that sensitivity of the cells of the anterior pituitary was affected following implantation with estrogenic compounds (Hongerholt et al., 1992).

Growth hormone does not act alone in eliciting observed responses following administration of estrogens. Trenkle (1997) summarized that GH initiates a signaling complex acting on the liver, which resulted in release of insulin-like growth factor-1 (IGF-1). Implanting cattle with estrogenic compounds was observed to increase the number of GH receptors on the liver (Breier et al. 1988a), which yielded higher levels of circulating IGF-1 (Breier et al., 1988b). Levels of IGF-1 were found to be amplified when estrogens were administered in combination with TBA (Johnson et al., 1996). Johnson (1998a) further explained these phenomena and

reported that IGF-1 mRNA levels in the liver were 150% greater in cattle implanted with a combination TBA/estradiol based implant. The same work demonstrated that localized production of IGF-1 mRNA at the level of the muscle cell was 68% higher in cattle implanted with TBA/estradiol. Further differentiation of the effects of these compounds was achieved by Pampusch (2008) who concluded that estradiol, and not TBA, was responsible for a localized increase in IGF-1 mRNA at the level of the muscle cell. Increased muscle mass observed following treatment with TBA/estradiol was explained by a 24% increase in proliferation of satellite cells in cultures isolated from implanted cattle relative to controls (Johnson et al., 1998b). Increased satellite cell proliferation was accompanied by increased numbers of myotube nuclei, which indicated that satellite cells were fusing with existing muscle cells and resulting in muscle cell hypertrophy (Johsnon et al., 1998b).

Satellite cells are responsible for post natal growth and are the source for 60 to 90% of DNA in the mature muscle fiber (Allen et al., 1979). Any modification to muscle cell growth must be achieved through hypertrophy and the relationship between growth factors; specifically IGF-1 and myogenic regulatory factors (MRFs) including Myo D, myf-5, myogenin and MRF-4. Insulin-like growth factor-1 has been reported to be involved with protein synthesis through many of the mechanisms described above (Johnson and Chung, 2007). However, other works have reported IGF-1 to be important to directing pluripotent cells toward the myogenic pathway and away from the formation of adipocytes (Johnson and Chung, 2007). Singh et al. (2003) reported that administration of testosterone and dihydrotestosterone to down-regulate important factors involved in adipocyte differentiation, specifically C/EBP-α and PPAR-γ. The mode of action of hormone-based implants is not fully understood, but these findings indicated that these

anabolic compounds up-regulate genes important to muscle development and down-regulate those involved in lipid formation.

Hormone-Based Implants – Antemortem Effects in Calf-Fed Holsteins

Effects of implants on calf-fed Holstein steers have been examined from the cellular level through application to large cattle feeding trials. Walker et al. (2007) determined the effect of TBA/estradiol-based implants on metabolism of calf-fed Holstein steers housed in metabolism crates. This work showed increased IGF-1 serum concentration and nitrogen retention following treatment with the combination implant. Localized IGF-1 mRNA expression in muscle cells of the loin tended to be higher in implanted cattle relative to non-implanted controls (Walker et al., 2007). This work indicated that a similar mechanism of action was responsible for muscle growth to that found in beef breeds. The earliest work that evaluated the effects of implants on calf-fed dairy steers utilized DES and was primarily performed in Canada. Forrest and Sather (1965) showed that gain was improved by up to 50% and that feed efficiency was improved in Holstein steers implanted at three different weights. This same work showed a 6% increase in feed consumption in implanted cattle relative to controls (Forrest and Sather, 1965). Forrest (1968) examined the effect of sex (bulls vs. steers) relative to the effectiveness of DES. This work determined that improvements in feed efficiency and gain were greater in implanted steers than in implanted bulls, with significant interaction between sex class and implant status (Forrest, 1968). Use of DES in Holstein bulls was further explored by Williams (1975) who found no difference or numerically-reduced indications of performance in feedlot bulls implanted with DES.

Use of a combination TBA/estradiol based implant was reported to increase gain by 17% in Holstein steers (Perry et al., 1991). The increased gain in calf-fed Holsteins was contrasted

with increases of 26% and 21% in Angus and crossbred steers within the same study (Perry et al., 1991). The combination implant (TBA/estradiol) also increased final weight, reduced days on feed, improved feed efficiency and increased DMI of Holstein steers (Perry et al., 1991). Apple et al. (1991) evaluated zearanol, progesterone, estradiol benzoate and TBA singularly and in combination in calf-fed Holstein steers. Over 249 d on feed, these workers found implants to improve ADG by 5 to 20%. Although not significant, DMI and feed efficiency were increased by up to 9% and 6%, respectively (Apple et al., 1991). Previous work showed that the greatest improvement in ADG and feed efficiency occurred in cattle implanted with TBA, followed by those treated with estradiol benzoate combined with progesterone (Apple et al., 1991).

Milton et al. (1998) examined the effect of combined implant protocols on calf-fed Holstein steers. Calves that were re-implanted once or twice with a TBA/estradiol implant were up to 6% more efficient compared to those cattle re-implanted with milder estrogenic compounds (Milton et al., 1998). Zinn et al. (1999) examined six different combinations of estradiol/progesterone-based implants combined with a purely TBA based product. This work resulted in the highest gains and greatest feed efficiency for calf-fed Holstein steers implanted with a TBA based product following use of an estradiol/progesterone combination implant (Zinn et al., 1999). A trial that compared TBA/estradiol-based implants to progesterone/estradiol products reported numerically, but non-significantly greater ADG in 850 lb Holstein steers given the TBA/estradiol combination implant (Kuhl et al., 1993). Use of a combination TBA/estradiol implant in Holstein steers raised under three separate feeding systems (grass-fed, pasture-supplemented, feedlot) resulted in a 13% increase in ADG across cattle under all management systems, with no interaction between management and implant status (Comerford et al., 2001). Repetitive use of TBA/estradiol based implants in calf-fed Holstein steers managed under

feedlot conditions for almost 300 d on feed was reported to increase final live weight by up to 9.8%, ADG by up to16.4%, and DMI by up to 8.9% in steers that received up to three implants during the finishing phase (Scheffler et al., 2003). Nearly linear increases in final live weight, ADG, and DMI were observed as steers were progressively implanted from zero to three times (Scheffler et al., 2003). Most recently, effects of implant strategy combined with and without use of the beta-agonist RH was explored by Bass et al. (2009). This work showed a 12 to 19% improvement in ADG of all implanted cattle relative to non-implanted controls, regardless of beta-agonist use (Bass et al., 2009). When used with RH, TBA/estradiol-based implants yielded the greatest improvement in ADG (Bass et al., 2009). Conversely, without supplementation of RH, estradiol/progesterone based implants produced the greatest response in ADG (Bass et al., 2009).

Hormone-Based Implants – Postmortem Effects in Calf-Fed Holsteins

Several studies concerning effects of hormone-based implants on carcass yield and meat quality have been conducted (Samber et al., 1996; Duckett et al., 1997; Roeber et al., 2000; Platter et al., 2003; Tatum, 2006; Schneider et al., 2007; Tatum et al., 2007). This review will focus on the effects of implants on carcass attributes of calf-fed Holstein steers. General effects were best summarized by Duckett et al. (1997) who suggested that all implants, except androgens alone, reduced marbling and percent of cattle that graded Choice. Duckett et al. (1997) also reported reduced external fat combined with increased HCW and REA as implant protocols became more aggressive. Tatum (2006) reviewed a variety of literature to determine that Warner-Bratzler shear force (WBSF) is increased up to 0.5 kg with increasing potency and use of implants.

The earliest evaluation of the effect of implants on cutability of Holstein steers was the work of Forrest (1968) who reported no effect of implants on dressing percentage or HCW; but significantly lower percent fat in carcasses from steers implanted with estradiol and progesterone. These findings were substantiated by later work that reported decreased fat and increased lean in Holstein steers treated with a combination estradiol/progesterone implant (Forrest, 1976). The same work found an increase in percent rump and hindquarter in Holstein cattle administered a combination estradiol/progesterone implant (Forrest, 1976). Evaluation of the effect of zeranol on Holstein steers concluded no significant difference in carcass lean, fat or bone between implanted cattle and controls (Ntunde et al., 1977). Forrest (1978) was unable to find significant differences in percentage of total carcass weight made up of any particular subprimal cut in Holstein steers implanted with progesterone and estradiol. The same work did find a lower percent fat and higher percent lean in carcasses from implanted cattle (Forrest, 1978). Administration of DES to Holstein bulls raised for beef production was found to increase HCW and REA, while reducing external fat (Williams et al., 1975).

Apple at al. (1991) found increases of 5 to 9% in HCW and 9 to 16% in REA, with no difference in dressing percentage, fat thickness, KPH or yield grade of Holstein steers implanted with zeranol or combination based implants. These findings agreed with those of Perry et al. (1991) who showed a 4% increase in HCW of Holstein steers implanted with a combination TBA/estradiol based product. The aforementioned work also evaluated change in percent of HCW comprised of subprimal cuts, but reported no differences between implanted cattle and controls (Perry et al., 1991). Thonney et al. (1991) reported numerically higher, albeit non-significant differences in HCW and REA of Holstein steers implanted with a combination TBA/estradiol implant. Evaluation of different combinations and numbers of implants on

cutability of calf-fed Holstein steers found numeric increases in HCW and REA of calves implanted with more aggressive TBA/estradiol based products (Milton et al., 1998). Further work found that repeated us of combination TBA/estradiol-based implants could increase HCW up to 10% and REA up to 11% when administered twice, or three times, compared to either once or never (Scheffler et al., 2003). Similar results were achieved by Cheatham and Duff (2004) who reported increases of 16% and 19% in HCW and REA, respectively when calf-fed Holstein steers were implanted three times with a combination of zeranol, estradiol or TBA/estradiol. A combination of various implant strategies with and without ractopamine hydrochloride found that only TBA/estradiol combination implants produced significant increases in HCW of calf-fed Holstein steers (Bass et al., 2009).

Preliminary research evaluating effects of implants on eating quality of Holstein steers found reduced sensory panel tenderness ratings for steaks derived from carcasses of steers treated with either DES or a combination of estradiol and progesterone (Forrest and Sather, 1965). The same work demonstrated lower overall panel scores for sensory attributes of steaks from implanted cattle (Forrest and Sather, 1965). Later work reported numerically, but not significantly lower sensory scores for all sensory attributes evaluated in steaks from steers implanted with a combination of estradiol and progesterone (Forrest, 1975). Ntunde (1977) reported non-significantly higher WBSF in steaks from steers implanted with zeranol.

More recent studies were conducted to evaluate the effect of different combinations of steroid hormones, administered either singly or in combination, on beef quality of calf-fed Holstein steers. Apple et al. (1991) reported numerically lower marbling scores and percent of cattle grading Choice or better in implanted cattle. The most extreme reductions in quality occurred in cattle treated with a combination of TBA/estradiol compared to controls (Apple et

al., 1991). The reduction in marbling score was approximately 16%, or nearly half a grade. However, these differences were not significant likely due to small sample sizes (n = 12) within each treatment (Apple et al., 1991). These findings are nearly identical to those of Perry (1991) who reported similar decreases in marbling; but again, this result was also non-significant due to small sample size (n = 12). In calf-fed Holstein steers implanted with a combination TBA/estradiol product at one of two initial weights (n = 16), no marbling differences were observed at lighter weights but reduced marbling scores were reported in cattle implanted at heavier weights (Thonney et al., 1991). The same work found contrasting results in quality grade; implanted cattle from the lighter weight group attained higher quality grades while those from the heavier group manifested lower quality grades (Thonney et al., 1991). Marbling scores approximately one-half quality grade lower have been observed in cattle implanted twice with a TBA/estradiol based products, following initial treatment with a progesterone/estradiol implant (Milton et al., 1998). These findings coincided with an over 20% reduction in percentage of cattle grading Choice (Milton et al., 1998), but differed from the findings of Scheffler et al. (2003) who reported only no difference in marbling between carcasses of non-implanted control steers and carcasses from calf-fed Holstein steers given either two or three TBA/estradiol-based implants. Kuhl et al. (1993) found that marbling score and percent Choice were numerically lower in calf-fed Holstein steers treated only once with TBA/estradiol implants compared with a single progesterone/estradiol based product. Inconsistent changes were observed for marbling score of carcasses from calf-fed Holstein steers treated with a combination progesterone/estradiol implant, following initial treatment with a zeranol-based product (Cheatham and Duff, 2004). These differences were contrary to the results of Bass et al. (2009) who found numerically and/or

significantly lower marbling scores, combined with increased skeletal and lean maturity in comparison of three different implant strategies to non-implanted controls.

Apple et al. (1991) found increased skeletal maturity in carcasses from cattle implanted with combination estradiol based products, and in those cattle implanted with a combination of TBA and zeranol. Increased skeletal maturity has been observed in carcasses from cattle implanted once, twice and three times with a TBA/estradiol product; however, those implanted a single time were not different from controls (Scheffler et al., 2003). Differences in marbling score, maturity and color are used as indicators of expected eating satisfaction. Apple et al. (1991) found no differences in sensory characteristics or WBSF of steaks derived from calf-fed Holstein steers administered one of five implant strategies, but overall tenderness tended to be lower in steaks from implanted cattle. These findings were similar to those of Perry et al. (1991) who found numerically, but not significantly, lower scores for all sensory attributes in steaks from steers treated with TBA/estradiol combination implants. Thonney et al. (1991) observed lower tenderness, juiciness and flavor in steaks from calf-fed Holstein steers implanted with a variety of protocols. Increased WBSF values have been reported in Holstein steers given multiple TBA/estradiol based implants (Scheffler et al., 2003). These results were similar numerically to WBSF of steaks from cattle implanted with zeranol followed by a progesterone/estradiol combination product (Cheatham and Duff, 2004).

Beta-Adrenergic Agonists – History

Beta-agonists belong to a class of compounds called phenethanolamines which are similar in structure to the naturally occurring catecholamines, epinephrine, norephinephrine and dopamine. Catechlomaines have been explored for over a century. Prior to discovery of all naturally occurring catecholamines, it was thought that two different receptors were involved in

binding these compounds. Workers theorized that these receptors may have different properties in different tissues (Dale, 1906; Robison et al, 1971). The possibility for use of beta- agonists to modify growth in livestock species traces to the 1960's when nicotine was administered to growing swine (Cunningham and Friend, 1967). Eventually, workers would identify clenbuterol as a metabolic modifier in livestock species, finding that the compound increased lean gain and reduced fat deposition (Ricks et al., 1984). Other compounds found to have similar effects included cimaterol, ractopamine and L-644,969 and zilpaterol (Bell et al., 1998).

Beta-Adrenergic Agonists – General Effects

The physiologic response following stimulation or inhibition of the receptors that bind synthetic or natural catcholamines are broad. These compounds have noticeable effects on the circulatory, digestive, muscular and endocrine systems. Consequently, beta-agonists have been evaluated within the realm of human medicine, specifically for treatment of asthma due to vasodilation that can occur following administration (Walker et al., 2007). Within livestock species, the effects of the natural and synthetic catecholamines on the digestive and endocrine systems are of greater interest due to the roles that they play in modification of animal growth. At the cellular level, catecholamines are responsible for activation of adenylate cyclase (AC) which results in production of cAMP. Cyclic-AMP is part of signaling mechanisms that elicit responses including gluconeogenesis by the liver, glycogenolysis in muscle and lipolysis in fat. Increased heart rate and blood flow coincide with increased contraction of skeletal muscle, which results in an increase in body temperature and respiration. All of these responses are typical to epinephrine and norepinephrine released as a result of stress (Gerrard and Grant, 2003).

Beta-agonists fed to livestock have been found to increase feed efficiency and ADG, while reducing DMI. Dramatic improvements in HCW and dressing percentage have been

reported as a result of beta-agonist use in cattle (Avendaño-Reyes et al., 2006; Scramlin et al., 2010). These effects, coupled with less significant changes in live weight, indicate a repartitioning of weight away from tissues removed at time of harvest and toward muscles that comprise the carcass. Effects postmortem include reduced carcass fat and increased skeletal musculature, particularly in the muscles of the hindquarter (Avendaño-Reyes et al., 2006). Marbling, tenderness and sensory attributes can all be negatively impacted by use of beta-agonists (Avendaño-Reyes et al., 2006; Dikeman, 2007; Scramlin et al., 2010; Arp, 2012). The ability of consumers to detect changes in sensory attributes of beef from cattle fed beta-agonits has not been conclusively proven, and may be dependent on the sample population to which the beta-agonists are applied; e.g. calf-fed Holstein versus beef breeds (Hilton et al., 2009; Mehaffey et al., 2009). The effect of beta-agonists on quality necessitates further exploration to determine best-management practices for all groups of cattle.

Beta-Adrenergic Agonists – Mode of Action

Understanding the mechanism by which beta-agonists cause change at the cellular level requires understanding of the receptor to which these ligands bind. The receptors binding the catecholamine epinephrine were first explored by Dale (1906) who demonstrated that the effects of epinephrine could be inhibited when ergo-toxin was used to block the receptor. Later work attempted to explain the effects of epinephrine using a single receptor model (Robison et al., 1971), and although this theory was proved incorrect, it was recognized that receptors for epinephrine were present in a wide range of tissues and caused a variety of reponses. Eventually, a dual receptor mode of action was proven by Ahlquist (1948). This work studied the response of several species and tissues to a variety of catecholamines and found that excitatory and inhibitory responses could result from stimulation with different catecholamines. This led to a

proposal for the classification of α and β receptors; a system that was later confirmed following discovery of β-blocking compounds (Robison et al., 1971). Both receptor subtypes are found throughout the body and have been classified based on the responses they cause. This review will focus on β_1 and β_2 receptors as these are primarily involved with binding of the commercially available beta-agonists used in livestock production. However, it should also be noted that considerable research has been conducted to evaluate the role of β_3 receptors, which are present in white and brown adipose tissue (Mersmann, 1998). Beta-3 receptors are unique in that several antagonists for the other two β receptor types are agonists for β_3 receptors. This could be a result of a distinctly different intracellular structure with regard to activation sites (Mersmann, 1998).

Beta-receptors belong to a superfamily called G-protein coupled receptors. G-protein coupled receptors (GPCR) are proteins composed of seven trans-membrane spanning domains that collectively form a pocket where ligands can bind. The carboxylic terminus of the receptor contains a serine/threonine rich region that is capable of binding with molecules essential to signaling pathways, in addition to providing a location for association with G-proteins. G-proteins are intermediaries in signaling pathways that allow receptors on the cell wall to bind with ligands and activate responses within the cell. A G-protein is made up of three subunits: α, β and γ which provide functional properties to the protein, as well as allow for classification. Regulation is initially dictated by binding of the guanine nucleotide guanosine triphosphate (GTP) to the α-subunit. The α subunit of G-protein associates with the receptor complex as well as binds and hydrolyzes GTP. The GTP molecule is hydrolyzed to guanosine diphosphate (GDP) and inorganic phosphate, which serves to down regulate the effector pathway. The disassociation of GDP acts as the rate limiting step within the signaling pathway. During

receptor activation, the G-protein binds the receptor as a heterotrimeric structure including all subunits (α, β, γ) of the protein. Guanosine diphosphate is subsequently replaced by GTP at which point the β and γ subunits dissociate from the complex. The α subunit/GTP complex is then capable of acting on effectors. Additionally, the β/γ dimer possesses the ability to act on either the same effector or a secondary effector. The α subunit possesses intrinsic GTPase activity and, following hydrolysis of GTP to GDP, the trimeric G-protein structure is reformed and the signaling cascade is terminated. The pathways best characterized as being stimulated by these mechanisms include those associated with retinal cyclic-GMP (light sensing) and AC.

Adenylyl cyclase is primarily activated by G_s (G-stimulatory) and inhibited by G_i (G-inhibitory). Binding of GTP to $G_{s\alpha}$ activates AC which is then capable of hydrolyzing ATP to cAMP. Cyclic-AMP can phosphorylate protein kinase-A which targets effectors within the cell. Hormone sensitive lipase (HSL) is one such target that serves as the rate limiting enzyme for triacylglycerol breakdown. Conversely, acetyl-CoA carboxylase, or the rate limiting enzyme for fatty acid synthesis is inactivated when phosphorylated. Protein kinase A is also capable of phosphoylating cAMP response element binding protein (CREB), which can in turn bind to the cAMP response element in a gene, causing transcription (Mersmann, 1998). A number of genes in the cell have demonstrated increased transcription following supplementation with beta-agonists, making this pathway a logical mode of action for these compounds (Mersmann, 1998).

Genes for expression of protein mRNA are up regulated following beta-agonist use. A portion of this work was carried out *in vitro* and it should be noted that these relationships may not be identical to those existing *in vivo*. Early work examining the effects of RH on gene expression showed either increased transcription of myosin light chain 1 and 3 or decreased protein degradation, which the authors summarized would increase protein synthesis (Smith et

al., 1989). Generally speaking, these findings were in agreement with many other investigations that utilized cimaterol and clenbuterol to demonstrate muscle cell hypertrophy along with increased calpastatin and decreased calpain I activity (Miller et al., 1988; Bardsley et al., 1992; Parr et al., 1992; Smith et al., 1995). Investigation into the effect of ZH on cell cultures found increased expression of IGF-1 and myosin heavy chain IIX mRNA (Tokach et al., 2011). Insulin like growth factor has been reported to increase protein accretion, while decreasing rate of protein degradation. Myosin heavy chain type IIX mRNA corresponds to larger, type II muscle fibers which are more sensitive to beta-agonists (Smith et al., 1995). This could explain the visible increase in muscle mass, particularly in the round, following administration of beta-agonists. *In vivo* models have found similar increases in MHC IIX mRNA; however, no change in IGF-1 expression (Rathmann et al., 2009; Baxa et al., 2010). The same work demonstrated a trend for increased calpastatin mRNA as the number of days that cattle were supplemented with ZH increased. Ractopamine hydrochloride has been shown to have either no effect on expression of IGF-1 mRNA (Grant et al., 1993; Walker et al., 2010). Walker et al. (2010) reported increased IGF Binding Protein-5 (IGFBP-5) mRNA in cattle treated with RH. Insulin-like growth factor binding protein-5 binds most IGF-1 in circulation and can either facilitate or inhibit binding of IGF-1 to the receptor. Expression of MHC IIX mRNA has been reported to be higher following administration of ractopamine to cull cows (Gonzalez et al., 2008).

Beta-Adrenergic Agonists – Implications of Change in Cellular Phenotype

Use of beta-agonists causes muscle cell hypertrophy and increased expression of type II muscle fiber types (Beermann et al., 1987; Kim et al., 1987; Miller et al., 1988; Vestergaard et al., 1994; Gonzalez et al., 2007, 2008; Kellermeier et al., 2009; Rathmann et al., 2009; Baxa et al., 2010). The importance of muscle fiber type and size to meat quality was best summarized by

Cassens (1977), who stated that "the properties of a muscle, be they visual appearance, physiological parameters or biochemical characteristics, are a reflection of the proportion of the muscle fiber types present." Ashmore et al. (1972) hypothesized that the quality of a meat product is a direct reflection of the proportion of fiber types present, continuing to describe that the selection of domesticated animals for increased meat yield may result in a transformation to a population with a greater percentage of large, white (type II) muscle fibers. The author stated that this selection may inadvertently promote alterations in the quality of meat products derived from domesticated animals (Ashmore et al., 1972). These impacts could negatively affect marbling, tenderness and color.

The theories of Ashmore et al. (1972) were explored by work that found a significant relationship between fiber type and tenderness ratings (Calkins et al., 1981). These workers found a significant negative correlation ($r = -0.40$) between percent of α-white (type II) muscle fibers and trained sensory panel tenderness ratings and significant positive correlation ($r = 0.44$) between percent of α-red fiber (type I) area and trained sensory panel ratings for tenderness. These findings were substantiated by the work of Kirchofer et al. (2002) who reported tenderness was reduced in muscles comprised of a greater amounts of white muscle fibers. Lipid content also was reduced in white muscle fiber types. These findings indicated potential for beta-agonists to negatively affect beef quality. Dikeman (2007) stated that beta-agonists must be used responsibly to avoid negative impacts on marbling and tenderness of meat products.

Beta-Adrenergic Agonists – Effect on Antemortem Performance

Beta-agonists are fed to cattle during the last 20 to 42 days of the finishing period to improve feed efficiency, ADG, dressing percentage and meat yields. In the U.S., RH is sold under the trade name Optaflexx® (Elanco Animal Health, Greenfield, IN) and can be

administered to steers and heifers during the last 28 to 42 days prior to harvest. The product is approved to be fed at a rate of 70 to 430 mg/head/d or 8.2 to 24.6 g/ton when used in a complete diet. When used as a top dress, 70 to 400 mg/head/d should be administered via an 800 g/ton blend fed at a rate of 1 lb/head/d. Optaflexx® has no withdrawal time prior to harvest, however export restrictions may exist on beef products from cattle fed RH. Zilpaterol hydrochloride, sold under the trade name Zilmax® (Merck Animal Health, Summit, NJ) is a beta-2 agonist that is typically fed to steers and heifers for the last 21 days on feed, but a 3 d withdrawal must be completed before harvest. Zilpaterol hydrochloride should be fed at a rate of 60 to 90 g/hd/d or 6.8 g/ton.

The use of beta-agonists in cattle has been evaluated in populations of continental European, British, crossbred, and to a slightly lesser degree, *Bos indicus* populations. General agreement exists that RH and ZH improve efficiency and ADG, while decreasing DMI (Avendaño-Reyes et al., 2006; Abney et al., 2007; Dikeman, 2007; Gruber et al., 2007; Sissom et al., 2007; Walker et al., 2007; Winterholler et al., 2007; Quinn et al., 2008; Vasconcelos et al., 2008; Beckett et al., 2009; Montgomery et al., 2009a, 2009b; Styrdom et al., 2009; Baxa et al., 2010; Bryant et al., 2010; Scramlin et al., 2010; Parr et al., 2011; Woerner et al., 2011; Arp, 2012; Jennings, 2012). These findings were reflected in a meta-analysis published by Elanco Animal Health (2012) that determined effects of RH on feedlot performance. In a summary of 32 studies, the authors reported a nearly linear increase in live weight gain as RH level was increased from 0 to 300 mg/hd/d. In cattle supplemented with RH at a level of 300 mg/hd/d, live weight was 10.2 kg greater, while ADG and feed efficiency were improved 20.5% and 16.4%, respectively. No effect on DMI was observed in the previous summary. A similar type of meta-analysis has not been published for the effects of ZH; however most work that has

compared ZH and RH within the same sample population have reported greater ADG and efficiency, coupled with reduced DMI in cattle fed ZH compared to RH (Avendaño-Reyes et al., 2006; Scramlin et al., 2010).

The body of literature surrounding the effects of beta-agonists on calf-fed Holstein steers is substantially more limited. No reports currently exist that have compared the effects of both RH and ZH within the same sample population of calf-fed Holstein steers. Work that investigated effects of RH fed at a rate of 200 mg/hd/d to calf-fed Holstein steers reported no difference in final live weight, feed efficiency or ADG (Bass et al., 2009). However, when fed at rates of 200 and 300 mg/hd/d within a different population of calf-fed Holsteins, steers supplemented with RH were 7 to 8 kg heavier at harvest, had a 17.5% increase in ADG and a 16.5% increase in feed efficiency, with no detectable differences in DMI (Vogel et al., 2009). Use of ZH for 20 d before harvest in sample populations of calf-fed Holstein steers has been reported to have no effect on ADG; however, feed efficiency was improved due to a strong trend for reduced DMI (Boler et al., 2009).

Contrasting bodies of evidence exist related to increases in final live weight following supplementation with beta-agonists. Some studies have reported no effect of ZH on final live weight (Beckett et al., 2009; Vasconcelos et al., 2008; Styrdom et al., 2009; Parr et al., 2011), compared to increased weights at time of harvest in populations of cattle supplemented with RH (Abney et al., 2007; Gruber et al., 2007; Winterholler et al., 2007; Vogel et al., 2009). However, not all research is in agreement with these findings (Quinn et al., 2008; Bass et al., 2009; Montgomery et al., 2009a, 2009b; Baxa et al., 2010; Bryant et al., 2010; Scramlin et al., 2010; Woerner et al., 2011). Research evaluating RH and ZH within the same sample population

found increased final live weights in beta-agonist treated cattle (Avendaño-Reyes et al., 2006; Scramlin et al., 2010).

Beta-Adrenergic Agonists – Effect on Carcass Yield

Beta-agonists increase HCW and improve dressing percentage. Quantifying this response and comparing the relative effects of the two commercially available beta-agonists has been the focus of considerable amounts of research. Unfortunately, very few of these studies have compared the two compounds head to head. Preliminary research conducted in Mexico on a diverse population of Charolais, Limousin, Brangus and Zebu influence cattle found that ZH administered at a level of 60 mg/hd/d increased HCW by 22 kg and dressing percentage by 2.0%, compared to RH at a level of 300 mg/hd/d that increased HCW 13 kg and dressing percentage 1.5% (Avendaño-Reyes et al., 2006). These results were comparable to those attained in evaluation of the effects of RH fed at 400 mg/hd/d and ZH at 60 mg/hd/d in another group of Mexican bred steers (Scramlin et al., 2010). This study reported no improvement in dressing percentage of steers supplemented with RH; however, ZH increased dressing percent approximately 2% (Scramlin et al., 2010). Hot carcass weight was greater following both treatments, with an increase of 6 kg and 13 kg for RH and ZH, respectively (Scramlin et al., 2010). Relative to final live weight, the improvement in HCW for cattle supplemented with ZH was actually greater than the increase in final live weight (Scramlin et al., 2010). These findings were significant in that they indicated weight not normally converted to HCW was somehow partitioned toward carcass tissues and away from non-carcass components. In the largest single study comparing RH and ZH within the same sample population, Arp (2012) used a sample population of steers in Texas to compare ZH fed at a rate of 6.8 g/ton and three levels of RH (200, 300, 400 mg/hd/d) to implanted controls. The findings of Arp (2012) were similar to those

of Scramlin et al. (2010) in that no effect of RH on dressing percentage was reported, while ZH increased dressing percentage by 1.4%. Hot carcass weight was 4 to 11 kg greater in RH 300/400 and ZH supplemented cattle (Arp, 2012). No difference in HCW was found between RH fed at a level of 400 mg/hd/d and the ZH treatment.

Studies that have evaluated only ZH have reported an increase of 5 to 21 kg in HCW, but most studies have reported increases over 10 kg in HCW and a 1 to 2.5 % increase in dressing percentage (Hilton et al., 2009; Montgomery et al., 2009a, 2009b; Baxa et al., 2010; Parr et al., 2011). These findings compare to those observed in RH supplemented cattle which have reported increases of 1 to 9 kg in HCW and 0.1 to 0.4% in dressing percentage (Abney et al., 2007; Gruber et al., 2007; Sissom et al., 2007; Walker et al., 2007; Winterholler et al., 2007; Quinn et al., 2008; Bryant et al., 2010; Woerner et al., 2011). Investigations into the effect of beta-agonists on sample populations of calf-fed Holstein steers have reported 3 to 5 kg increases in HCW and non-signficant differences in dressing percentage of steers fed ractopamine at 200 and 300 mg/hd/d (Bass et al., 2009; Vogel et al., 2009). Zilpaterol hydrochloride fed at a level of 6.8 g/ton to calf-fed Holstein steers for 20 d prior to harvest was reported to increase HCW approximately 12 kg and dressing percentage by nearly 1.6% (Beckett et al., 2009). This limited body of work surrounding use of beta-agonists in calf-fed Holstein steers indicates that effects may be similar to those observed in beef breeds.

Increased muscle as a result of beta-agonist use has been documented through increased ribeye area (REA) and quantified through subprimal cutout yield analysis. Preliminary research on the effect of beta-agonists on livestock species reported use of clenbuterol, cimaterol and L-644,969 to increase the area of the LM. Ricks et al. (1984) found increases in LM area of 7.0 cm^2, 7.0 cm^2 and 13.0 cm^2 following administration of clebuterol to lambs, swine and cattle,

respectively. The increase in muscle was accompanied by reductions in rib fat thickness (Ricks et al., 1984). These findings were similar to those from studies that evaluated the effect of cimaterol or L-644,969 on Holstein cattle (Moloney et al., 1990; Chikhou et al., 1993a, 1993b; Moloney et al., 1994; Vestergaard et al., 1994).

Recent research has determined the effect of ZH and RH on muscle and fat content of beef breeds. Head to head comparisons of RH and ZH have found that both compounds increase LM area with variable effects on fat thickness. Initial work that evaluated RH at a level of 300 mg/hd/g and ZH at 60 mg/hd/d found an increase in LM area of 5 cm^2 as a result of RH and 8 cm^2 as a result of ZH (Avendaño-Reyes et al, 2006). Fat thickness between the 12^{th} and 13^{th} rib was unaffected by RH, but ZH was associated with a reduction of 0.3 cm (Avendaño-Reyes et al., 2006). The previous work fully deboned carcasses and determined that percent lean was 1.7% and 2.2% higher in carcasses from the RH and ZH treatments, respectively (Avendaño-Reyes et al., 2006). No differences in percent fat or bone were detected as a result of either beta-agonist (Avendaño-Reyes et al., 2006). When RH was supplemented at a rate of 400 mg/hd/d and ZH at 60 mg/hd/d, Scramlin et al. (2010) found a 3.7 cm^2 increase in LM area of cattle supplemented with ZH; no changes were observed in REA of steers treated with RH. Last rib fat was lower in the ZH treatment, along with less KPH, which resulted in an average yield grade of 2.3 in ZH treated cattle compared to 2.9 for RH and control treatments (Scramlin et al., 2010). The previous work evaluated cutability through a carcass cutout and determined carcass fat was 1% lower in ZH treated cattle (Scramlin et al., 2010). In ZH treated cattle, cuts from the round and loin comprised a substantially greater percentage of total carcass weight compared to the RH and control treatments (Scramlin et al., 2010). Arp (2012) reported similar results in that cattle supplemented with RH at levels less than 400 mg/hd/d had no difference in REA compared to

implanted controls. Ractopamine hydrochloride at a level of 400 mg/hd/d and ZH increased LM area by 2.4 cm^2 and 6.7 cm^2, respectively (Arp, 2012). The previous work reported no difference in 12^{th} rib fat thickness or yield grade as a result of beta-agonist supplementation. However, total saleable yield was 0.7% and 1.1% higher in carcasses from cattle treated with RH at a level of 400 mg/hd/d and ZH, respectively (Arp, 2012). The work of Arp (2012) agreed with that of Scramlin et al. (2010) in that beta-agonists caused the greatest increases in percentage of total side weight comprised of muscles of the round and loin.

Works that have not evaluated both commercial available beta-agonists in the same sample population are in relative agreement with the previously cited studies concerning the increase in muscle and decrease in fat as a result of beta-agonist use. Rathmann et al. (2009) found increased cutability in carcasses of cattle supplemented with ZH and noted that the most dramatic changes occurred in muscles of the round. Similar results were achieved by Kellermeier et al. (2009) who reported an increase in HCW of 15 kg, and a 14% increase in REA of cattle fed ZH. Leheska et al. (2009) showed increased side weight coupled with reduced body fat following administration of ZH for 20 and 40 d before harvest. Existing literature has generally found an increase in LM area of 5 to 9 cm^2, a decrease in last rib fat of 0 to 0.2 cm and 0 to 0.2% less KPH in carcasses from cattle fed ZH for 20 d (Vasconcelos et al., 2008; Hilton et al., 2009; Montgomery et al., 2009a, 2009b; Baxa et al., 2010; Rathmann et al., 2012). These effects compare to RH supplemented cattle that have been found to have a 2 to 3 cm^2 larger REA, with most works finding no effect of RH on last rib fat or KPH (Abney et al., 2007; Gruber et al., 2007; Sissom et al., 2007; Quinn et al., 2008; Bryant et al., 2010; Woerner et al., 2012).

Specific to calf-fed Holstein steers, improvements in cutability following beta-agonist use have been documented in populations fed RH or ZH, but have never been explored in a

contemporary group fed both compounds. Use of RH at 200 or 300 mg/hd/d was reported to increase in REA 1 to 3 cm^2 (Bass et al., 2009; Vogel et al., 2009). The work of Vogel et al. (2009) found modest reductions in last rib fat (-0.08 cm) when RH was supplemented at a rate of 300 mg/hd/d, however both Vogel et al. (2009) and Bass et al. (2009) found no differences in fat thickness of calf-fed Holstein steers supplemented with RH at 200 mg/hd/d. Neither of the aforementioned works found differences in KPH due to RH supplementation. Use of ZH in populations of calf-fed Holstein steers has been reported to increase REA 3 to 8 cm^2, with no effect on last rib fat thickness or KPH (Beckett et al., 2009; Garmyn et al., 2010; Hosford, 2010). Work that has evaluated the effect of ZH on subprimal yield of calf-fed Holstein steers found improved cutability in cattle fed ZH for 20 d, with substantial increases in percent of HCW comprised of cuts of the round and loin, as well as the ribeye roll (Boler et al., 2009). The previous work reported a 1.2% increase in percent saleable yield following treatment with ZH. Further anlaysis of subprimals from the same sample population revealed percent fat to be numerically lower in the *Triceps brachii*, *Gluteus medius* and *Longissimus lumborum* from steers fed ZH (Holmer et al., 2009). These findings were in agreement with those of Garmyn et al. (2010) who found increased subprimal yield, as well as decreased percent fat and bone in carcasses of Holstein steers supplemented with ZH. The previous work found ZH to increase saleable yield by 2.7% compared to controls. Percent of HCW comprised of individual subprimal cuts was similar to previous research in that cuts of the round and loin were found to have the greatest response to ZH treatment. Despite these findings, those of Hankelhaus et al. (2011) determined that the cutability advantage gained from use of ZH was primarily achieved in the carcass to subprimal cutout, but not in the subprimal to retail conversion. This indicated that

beta-agonists add weight; however, the retailer may be purchasing cuts that are simply heavier, not more profitable in terms of retail yield.

Beta-Adrenergic Agonists – Effect on Meat Quality

Beta-agonists negatively impact most traits associated with beef quality. The majority of research available on this topic reported decreased marbling and/or tenderness following administration of either RH or ZH. Early work that evaluated effects of beta-agonists on intramuscular fat failed to find a difference in marbling score of steers treated with clenbuterol (Ricks et al., 1984). However, the work of Miller et al. (1988) showed that use of clebuterol decreased intramuscular lipid cell size and activity of lipogenic enzymes in heifers. Chikhou et al. (1993b) reported an increase of up to 118% in shear force following administration of cimaterol to Holstein steers. The beta-agonist L644-969 was found to reduce intramuscular lipid by up to 50%, while increasing shear force over 200% when administered to Holstein steers (Moloney et al., 1994). Dikeman (2007) summarized that clenbuterol and cimaterol both caused negative impacts on intramuscular fat and tenderness.

The beta-agonists used in the U.S. today have not been shown to be as detrimental to quality as those first explored for use in livestock production. Nevertheless, differences exist when comparing effects of RH and ZH to controls, as well as against one another. It is generally accepted that the impacts of RH on meat quality are less significant than those of ZH. However, it should be recognized that as dosage level of RH has been increased, a nearly linear reduction in many of the important meat quality attributes has been observed (Elanco, 2012). The work of Scramlin et al. (2010) found no differences in marbling score between beta-agonist treatments and controls; however this sample population was exceptionally high quality with average initial marbling scores equal to mid-modest. Several studies have compared RH and ZH together and

found an increase in shear force values associated with beta-agonist supplementation. Ractopamine hydrochloride was reported to increase WBSF by 0.5 kg or less, while ZH increased WBSF by approximately 1.8 kg (Avendaño-Reyes et al., 2006; Scramlin et al., 2010). Some work has reported a more moderate effect of beta-agonists on quality, with reductions of approximately 20 units in marbling following administration of RH (300 mg/hd/d) and ZH (Arp, 2012). The previous work found no decrease in percent Choice as a result of beta-agonist use; however, a numeric decrease of approximately 10% was reported between the control and ZH treatments (Arp, 2012). Within the same sample population, WBSF was increased 0.3 kg and 0.6 kg in RH and ZH treatments, respectively (Arp, 2012).

In studies that have evaluated the effect RH alone, most have reported a decrease in marbling score of 0 to 30 units, a decrease in percent Choice of 0 to 5%, an increase in WBSF value of 0 to 0.4 kg and a decrease of just over 10% in panel ratings for tenderness (Abney et al., 2007; Sissom et al., 2007; Gruber et al., 2007; Quinn et al., 2008; Gonzalez et al., 2010; Woerner et al., 2012). Several studies have found reductions in marbling score of up to half a degree of marbling (≈ 50 units) following RH supplementation (Walker et al., 2007; Winterholler et al., 2008). Most workers have reported ZH to reduce marbling 30 to 50 units, decrease percent Choice by approximately 10% and increase WBSF 0.5 to 1.5 kg dependent on age postmortem (Vasconcelos et al., 2008; Brooks et al., 2009; Montgomery et al., 2009a; Kellermeier et al., 2009; Hilton et al., 2009; Rathmann et al., 2009; Garmyn et al., 2011; Rathmann et al., 2012). It should be noted that several studies have found increased drip loss associated with storage of beef products derived from cattle that have received beta-agonists (Avendaño-Reyes et al., 2006; Rathmann et al., 2009). This could be significant considering complaints within the beef industry associated with subprimal purge, particularly in cuts from the round.

Specific to calf-fed Holstein steers, workers have reported modest decreases in marbling score (≈ 10 units) of carcasses from cattle treated with RH (Bass et al., 2009; Vogel et al., 2009). Vogel et al. (2009) reported variable effects in percent of carcasses grading Choice between controls and those treated with RH at 200 or 300 mg/hd/d. No shear force or tenderness observations were collected as part of the previously mentioned works. Use of ZH in calf-fed Hostein steers was reported to decrease marbling score by approximately 20 degrees and percent Choice by 7 – 10% (Beckett et al., 2009). These results were in disagreement with those of Garmyn et al. (2010) who reported no difference in marbling scores in calf-fed Holstein steers that were treated with ZH.

Tenderness has been thoroughly explored in products derived from calf-fed Holstein steers. Most of these works cite steaks from ZH treated cattle to have 14 d WBSF values approximately 1.0 kg higher than controls, but this value is typically reduced approximately 0.5 kg by 21 d postmortem (Holmer et al., 2009; Mehaffey et al., 2009; Garmyn et al., 2010). Trained panelists have been able to detect differences in steaks from Holstein cattle fed ZH before harvest; typically rating samples lower for overall tenderness (Hilton et al., 2009; Garmyn et al. 2010). These differences have been found to be detectable by some consumer panels at 14 d postmortem (Mehaffey et al., 2009), but Hilton et al. (2009) reported no difference in consumer panel ratings for tenderness of steaks from ZH treated cattle. The work of Mehaffey et al. (2009) found similar results once steaks reached 21 d postmortem. Conflicting results related to the ability of consumers to detect differences in tenderness and overall desirability of steaks from calf-fed Holsteins supplemented with ZH compared to steaks from control steers fed no beta-agonists prompts the question as to whether inherently higher quality cattle may be able to

be treated with more aggressive growth promotants without detectable changes in sensory attributes.

Summary

Calf-fed Holstein steers pose potential problems to feedlot producers in terms of efficiency (Fox et al., 1988; Duff and McMurphy, 2007) and to beef processors due to low dressing percentage and muscle to bone ratio (Schaefer, 2005). Ionophores and growth promotants such as hormone based implants and beta-agonists could address these issues (Bergen and Bates, 1984; Duckett, 1997). Dikeman (2007) summarized growth promotants must be used judiciously to avoid detrimental effects on meat quality. Differences exist between the commercially available implants and beta-agonists relative to their effects on beef quality (Avendaño-Reyes et al., 2006; Tatum, 2006; Scramlin et al., 2010; Arp, 2012). Calf-fed Holstein steers are inherently high quality (Schaefer, 2005), consequently they may be able to be treated with more aggressive growth promotants without detectable differences in sensory attributes (Hilton et al., 2009; Mehaffey et al., 2010). However, no comprehensive study has evaluated the effect of both commercially available beta-agonists in the same population of calf-fed Holstein steers. Growth promotants are essential to profitability of the beef industry (Lawrence and Ibarburu, 2007), but their impact on quality and consumer acceptability must be quantified in all cattle populations to allow producers to exceed the current level of beef quality at retail.

CHAPTER III

NORTH AMERICAN BEEF TENDERNESS SURVEY 2011-2012: BENCHMARKING TENDERNESS AND SAMPLE SHIPPING PROCEDURES

INTRODUCTION

Beef tenderness has been one of the most thoroughly investigated topics in the field of meat science. Summaries exist which have addressed pre-harvest influences (Tatum, 2006, 2007), post-harvest interventions (Smith et al., 2008) and prediction of beef tenderness (Woerner and Belk, 2008). Beef tenderness has been monitored through national surveys of retail locations (Morgan et al., 1991; George et al., 1999; Brooks et al., 2000; Voges et al., 2007; Savell, 2012). National beef tenderness surveys have demonstrated a trend for improved tenderness over the time (Table 1). Despite this progress, the beef industry faces declining consumer demand (LMIC, 2013) in a market place that has demonstrated increased willingness-to-pay for more tender, or guaranteed tender products (Boleman et al., 1997; Platter et al., 2005). This contrast necessitates evaluation of the systems utilized to determine the acceptability of the tenderness of beef products at the consumer level.

Industry reliance on surveys to benchmark and monitor tenderness requires precision and accuracy, while being able to quantify the effect of protocol on tenderness observations. The influence of freezing, thawing, cooking and coring on Warner-Bratzler shear force (WBSF) have been examined (Parrish et al., 1973; Wheeler et al., 1994, 1996; Wulf et al., 1996, Shanks et al., 2002). Standardization of shear force protocol addresses a significant portion of potential variation between the reported results of the aforementioned studies. However, tenderness surveys require that all samples be somehow exposed to shipping conditions before shear force

determination, a practice that could influence tenderness observations. The effect of shipping and handling on shear force has never been quantified. The survey portion of this work was conducted to benchmark and monitor beef tenderness; data from the survey portion of this work demonstrated the need to quantify the effect of shipping on WBSF and slice shear force (SSF) of beef top loin steaks.

MATERIALS AND METHODS

Tenderness Survey

Sample Collection

Thirty cities in the U.S. and Canada were identified as potential sampling sites. Cities were selected within one of six geographic regions (Northeast, Southeast, Midwest, West, Texas and Canada). Cities sampled were, Albuquerque, NM; Atlanta, GA; Billings, MT; Birmingham, AL; Boise, ID; Boston, MA; Charlotte, NC; Chicago, IL; Columbus, OH; Washington, DC; Des Moines, IA; Detroit, MI; Fayetteville, AR; Fort Collins, CO; Fort Worth, TX; Kansas City, KS; Las Vegas, NV; Los Angeles, CA; Miami, FL; Minneapolis, MN; Nashville, TN; New Orleans, LA; New York City, NY; Philadelphia, PA; Phoenix, AZ; Pittsburgh, PA; Portland, OR; Reno, NV; San Antonio, TX; San Francisco, CA; Seattle, WA; St. Louis, MO; Tampa Bay, FL; and Winnipeg, ON, CA. Retailers in each city were identified based on regional market share (Supermarket News, 2013). Supermarket News (2013) was utilized to insure representation of most major retailers in North America. Number of stores sampled per city was weighted based on census data for the greater metropolitan area. Two to five retailers were sampled in all locations. Personnel from Elanco Animal Health were identified in each location and trained to conduct the sampling procedures. A minimum of two top loin and two sirloin steaks were

collected each month from each retail location. Samples reduced in price for quick sale were avoided; otherwise collection was random with no preference for cut-style, weight, dimensions, quality grade or other marketing claims. Location in the retail also was totally random. Samples were collected over twelve months starting in June 2011.

Shipping and Handling

Samples were transported from retail locations in styrofoam coolers containing ice packs. Two sizes of cooler (45.7 x 45.7 x 40.6 cm or 45.7 x 30.5 x 30.5 cm) were used based on number of samples collected. Samples were shipped in materials used for retail display, stacked in the bottom of coolers with ice packs on top or card stacked with ice packs in between. Within 24 h of purchase, samples were shipped via overnight air delivery to Colorado State University, Fort Collins, CO. During NABTS - Period 1 (June 2011-Nov 2011), samples were shipped fresh from point of origin and frozen on arrival. This protocol was amended during NABTS - Period 2 (Dec 2011-May 2012) due to the effect that shipping appeared to have on shear force values. Methodology used to quantify this effect is described below. During NABTS - Period 2 samples were frozen for 24 – 48 h in home refrigeration units before shipment to Colorado State University. The change in protocol caused some cities to be removed from the survey due to limitations associated with space in home refrigeration units. Period 1 included N=586 top loin and N=518 sirloin steaks, compared to N=394 top loin and N=342 sirloin steaks in Period 2.

Receiving of Samples

Samples were evaluated for temperature upon arrival at Colorado State University. Samples unacceptable for surface temperature (NABTS-Period 1 > 7° C, NABTS-Period 2 > 0° C) were removed from the survey population. Packaging material was cataloged and marketing claims, price, weight, store and location were recorded. During NABTS-Period 1, sample

dimensions were obtained on arrival, prior to freezing. The protocol used during NABTS-Period 2 resulted in samples arriving frozen, consequently dimensional data was collected immediately before shear force determination, after samples had been thawed. All samples were transferred from retail packaging, vacuum packaged and frozen at -28.8° C.

Warner-Bratzler Shear Force Determination

Samples were thawed at for 24 h at 4° C to a target pre-cooking internal temperature of 1 to 3° C. Cooking was achieved using a GRP99 Next Generation Grill (Spectrum Brands Inc., Madison, WI). Samples were cooked to a target peak internal temperature of 71.1° C and allowed to cool to room temperature for 4 h before shear force determination. Warner-Bratzler shear force analysis was conducted according to the guidelines of AMSA (1995) on an Instron Universal Testing Machine, Model 4443 (Instron Corporation, Canton, MA) with a cross-head speed of 200 mm/min.

Trained Sensory Panel Evaluation

Panelists (n=10 per period) were trained for evaluation of 9 sensory attributes including myofibrillar tenderness, connective tissue tenderness, overall tenderness, juiciness, beef flavor, buttery, metallic, livery, grassy, oxidized, saline and soapy. Training was conducted using beef *Longissimus dorsi, Biceps femoris, Gluteus medius, Infraspinatus, Semitendinosus* and *Psoas major.* Flavor profile training was conducted using the standards published by (Adhikari et al., 2011) and through use of steaks enhanced with saline solution. All samples remained frozen for at least 1 wk prior to trained panel evaluation. Within each panel, top loin and sirloin steaks from adjacent months were represented. Thawing and cooking were conducted in a manner identical to that described for shear force determination. Samples were trimmed of external edges and connective tissue prior to being portioned into sensory samples (1.27 x 1.27 cm).

Sensory samples from vein steaks were only derived from portions of the *Longissimus dorsi*; sirloin samples were derived only from the *Gluteus medius* when possible. Panelists were served two cubes under red-lighting and provided with unsalted crackers, apple juice and distilled water to cleanse the palate. Responses were marked on a 15 cm continuous line scale. Tenderness attributes, juiciness and beef flavor were marked for all samples. Panelists had the option to write in up to three off flavors from those described above and mark their presence on three additional 15 cm lines provided at the bottom of the ballot.

Statistical Methods

Statistical analysis was conducted using SAS 9.3 (SAS Institute Inc., 2012). Summary statistics were compiled using the MEANS and FREQ procedures. Regression of panel responses against WBSF values was conducted to determine panel responses most correlated to tenderness thresholds of 4.4 kg (Platter et al., 2005; ASTM, 2011). During Period 1, this value was 7.5 cm; during period 2, this value was 8.1 cm.

Effect of Shipping on Shear Force

Collection of Samples

Three cases of USDA low Choice strip loins were collected directly from the packaging line in a commercial processing facility in Colorado. Loins (approx. 6.8 kg) were removed from the box and transported in coolers on ice to Colorado State University, Fort Collins, CO. Upon arrival, loins were re-packaged into their original boxes for aging at 4° C for 14 d from the box packing date. Eighteen loins were selected at random for fabrication into top loin steaks. Each loin was divided into three sections (anterior, medial and posterior) from which four 2.54 cm thick steaks were cut. Steaks from within each section were randomly assigned to one of four treatments: (1) shear fresh; (2) shear following freezing; (3) display 68 h in a retail case, freeze

and shear; (4) display 68 h in a retail case, expose to either simulated shipping (Trial 1) or actual shipping (Trial 2), freeze and shear. Steaks assigned to treatment two, three and four were placed into styrofoam trays and overwrapped using polyvinylchloride packaging. Following packaging, steaks assigned to treatment two were frozen at -28.8° C. Steaks assigned to treatments three and four were placed in a cooler and transported on ice to an offsite location for retail display. Steaks were randomized in a 1.8 m wide, three layer, fluorescently lit Hussmann® display case. During Trial 2, temperatures in the retail case were recorded every ten minutes using six Escort iLog Data Loggers (Escort Data Loggers, Inc., Buchanan, VA).

During both trials, only the top two shelves of the case were used due to sample numbers and the inability to appropriately fill all three layers in a manner similar to commercial retail displays. Following display, steaks were transported in coolers, on ice to Colorado State University and steaks assigned to treatment three were frozen in a manner identical to that described above.

During Trial 1, steaks assigned to treatment four were placed in styrofoam coolers (45.7 x 45.7 x 40.6 cm) that contained ice packs, boxed and then placed on the back of a cargo truck for 24 h to simulate shipping. During Trial 2, steaks assigned to treatment four were transferred directly from the retail case, randomly assigned to one of five styrofoam coolers (45.7 x 45.7 x 40.6 cm) containing ice packs, boxed and shipped via overnight air delivery to Oklahoma State University, Stillwater, OK. Head space in the coolers was filled with butcher paper and a temperature logger was fixed to the cooler lid. Upon arrival at Oklahoma State University, samples were unpacked and frozen in a manner similar to that described above. Steaks remained frozen at Oklahoma State University for approximately 1 wk before being returned via shipment to Colorado State University. Upon arrival, all samples were placed into storage at -28.8° C.

Approximately 3 d later, samples from treatments two, three and four were removed from overwrap packaging and vacuum packaged.

Shear Force Measurements

Thawing and cooking were conducted in a manner identical to that describe above. Slice and Warner-Bratzler shear force were conducted simultaneously on all steaks from anterior and medial sections of each loin using methodology described by Lorenzen et al. (2010). Steaks from posterior sections were assessed using WBSF only. Warner-Bratzler shear force analysis was conducted according to the guidelines of AMSA (1995). All tests were conducted on an Instron Universal Testing Machine, Model 4443 (Instron Corporation, Canton, MA). Warner-Bratzler shear force testing was conducted using a cross-head speed of 200 mm/min; SSF determination was conducted with a cross-head speed of 500 mm/min.

Statistical Methods

Statistical analysis was conducted using SAS 9.3 (SAS Institute Inc., 2012). Normality of shear force data was determined using the W-statistic and distribution of variance was evaluated using a plot of residuals. Main effects and interactions were tested in mixed models (PROC MIXED) that contained the fixed effect of treatment and the random effects of individual loin and loin section (anterior, medial, posterior). General mixed linear models (PROC GLIMMIX) that had identical fixed/random effects to those summarized above were used to find the probability of tough steaks as a result of treatment. The Newton-Rhapson optimization method was used in general mixed liner models. Denominator degrees of freedom were calculated using the Kenward-Roger method (Kenward and Roger, 1997) for all models. Means were separated using pairwise t-tests and a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

North American Beef Tenderness Survey Results

Period 1 – Samples Shipped Fresh, Frozen on Arrival

Mean WBSF values from the North American Beef Tenderness Survey (NABTS) are presented in Table 3.1. Top loin steak WBSF data were most similar to the findings of Brooks et al. (2000), whereas sirloin steak data were most comparable to the results of Morgan et al. (1991) and George et al. (1999). Mean WBSF for top loin and sirloin steaks were dramatically higher than recent National Beef Tenderness Surveys (Voges et al., 2007; Savell, 2012). Comparisons between survey data may not be valid as differences in protocol existed between many of the aforementioned works. The protocol of Morgan et al. (1991) used a target internal cooked temperature of 65° C for WBSF determination, compared to all other works that used target peak internal temperatures of approximately 70° C. Brooks et al. (2000) wrapped steaks in Saran film immediately following cooking while tempering at 4° C for 10 h before WBSF determination. This tempering time differed substantially from the 4 h at room temperature used by Voges et al. (2007) and the present work. Variations in shear force protocol are influential to WBSF observations (Wheeler et al., 1996).

Frequencies of WBSF observations greater than 3.9 kg are reported in Table 3.1. The threshold of WBSF ≥ 3.9 kg allowed comparison between major tenderness surveys as all works reported frequencies above and below this mark. Data from NABTS-Period 1 are in general agreement those of George et al. (1999), but had a higher incidence of steaks with WBSF ≥ 3.9 kg compared to other National Beef Tenderness Surveys (Brooks et al., 2000; Voges et al., 2007; Savell, 2012). Sirloin steak data had a frequency of samples categorized as intermediate or tough that was 10% higher than any previous survey. Frequency distributions for WBSF of top

loin and sirloin steaks by period are presented in Figure 3.1 and Figure 3.2, respectively. Shear force data from NABTS-Period 1 reflected a heavy right-hand skew with a high number of observations below 2.0 kg. This represented significant deviation from previous works that have used similar shear force protocols (Platter et al., 2005; Gruber et al., 2006). These observations were noted in the first three months of data collection during completion of the present study, prompting exploration of the effect of shipping on WBSF values. Results of an informal study conducted from September 2011 to November 2011 suggested an approximate increase of 0.50 kg in WBSF of samples shipped frozen versus those shipped fresh and frozen on arrival. During NABTS-Period 2, samples were shipped frozen from point of origin. A designed experiment was conducted to quantify the effect of shipping on shear force observations, this work is summarized below.

Period 2 – Samples Shipped Frozen, Stored Frozen

Means for WBSF data from NABTS-Period 2 data are reported in Table 1. The differences in WBSF data between NABTS-Period 1 and Period 2 (≈0.5 kg) were nearly identical to those differences observed in informal work. Top loin and sirloin sample populations had higher mean WBSF values than had been observed in any previous U.S. beef tenderness survey. Means for WBSF of top loin steaks from NABTS-Period 2 were more similar to the findings of multiple non-survey based works from a variety of institutions; these studies have reported mean WBSF values for top loin steaks between 2.6 and 4.4 kg at either 14 or 21d postmortem when samples were sourced from a variety of quality grades and frozen (Shackelford et al., 1995; Belew et al., 2003; Lorenzen et al., 2003; Platter et al., 2005). Top loin steaks shipped frozen (NABTS-Period 2) displayed a frequency distribution for WBSF that was similar to that observed by Platter et al. (2005). Several of the previously mentioned works also

reported WBSF data for sirloin steaks that were similar to that observed in the current work. North American Beef Tenderness Survey data from Period 2 found mean WBSF = 4.0 kg for sirloin steaks, which is ± 0.5 kg from those means published by Shackelford et al. (1995) and Belew et al. (2003). When compared to the work of Gruber et al. (2006) who evaluated WBSF of fresh steaks, NABTS-Period 2 means for top loin and sirloin steaks are approximately 0.5 kg lower which would roughly correspond to the effect of freezing demonstrated by Shanks et al. (2002). The postmortem age of samples in tenderness surveys is likely substantially more variable than that in controlled studies. However, the most recent National Beef Tenderness Survey published a mean aging time of 21.6 d for top loin steaks with a trend for more cuts at retail to be less than 14 d postmortem compared with 2005/2006 National Beef Tenderness Survey data (Voges et al., 2007; Savell, 2012), indicating comparisons to works using 14 and 21 d aging times may be relatively acceptable.

The frequency of top loin and sirloin steaks categorized as either intermediate or less than tender during NABTS-Period 2 was greater than similar frequencies from any previous survey work. The change in shipping protocol from NABTS-Period 1 to Period 2 normalized the distribution of top loin steak shear force values (Figure 3.1). The frequency distribution of shear force values for sirloin steaks from NABTS-Period 2 displayed similar curvature to that from NABTS-Period 1 data; however across all segments of the distribution, the mean, median and mode observations were approximately 0.5 to 1.0 kg greater (Figure 3.2). The magnitude of this difference was identical to the shift in mean, median and mode shear force within the sample population of top loin steaks (Figure 3.1).

Panel Data

Means for panel responses by period are reported in Table 3.2. All panelists were trained; however, experience of panelists during Period 1 was approximately 3 to 5 years, compared to 1 to 3 years during Period 2. Correlation coefficients between WBSF and overall tenderness ratings were -0.55 and -0.45 during NABTS-Period 1 for top loin and sirloin steaks, respectively. This compared to -0.42 and -0.45 during NABTS-Period 2 for top loin and sirloin steaks, respectively. During NABTS-Period 2, WBSF suggested that 14.3% of top loin samples were slightly tough (WBSF $\geq$ 4.4 kg). Sensory panel data from NABTS-Period 2 found 20.3% of top loin steaks had mean overall tenderness ratings of less than 8.1 cm, which was approximately equal to 4.4 kg of WBSF as determined by regression analysis. Period 1 data suggested that 8.1% and 15.4% of steaks were slightly tough based on WBSF and trained sensory panel determination, respectively. During NABTS-Period 1, a mean panel rating for overall tenderness of 7.5 cm equaled 4.4 kg of WBSF based on regression analysis. Determination that trained panel responses equivalent to WBSF of 4.4 kg were approximately equal to half the distance of the 15 cm line scale seems appropriate as the designation of "slightly tough" has been reported halfway between 1 and 8 on hedonic systems used in trained panels during previous tenderness surveys (Morgan et al., 1991; George et al., 1999).

Effect of Shipping on Shear Force

Trial One – Simulated Shipping Conditions

Freezing, retail display and simulated shipping had varying effects on shear force values depending on WBSF or SSF data (Table 3.3). Freezing resulted in lower WBSF values ($P < 0.05$) which agrees with the findings of Shanks et al. (2002). Differences in WBSF were observed as a result of retail display ($P < 0.05$), but no difference was found as a result of

simulated shipping ($P > 0.05$). These findings were contrasted by SSF data in which the only difference was found to be reduced shear force as a result of simulated shipping ($P < 0.05$). Comparison across treatments must consider some influence of aging on shear force since samples displayed and shipped were 3 – 4 d older than those sheared fresh. However, the work of Gruber et al. (2006) found the majority of aging was completed in upper two-thirds Choice top loin steaks by 15 d postmortem. These samples were low Choice, but using the models published by Gruber et al. (2006), the aging response between 14 and 18 d postmortem was estimated to be 0.0 – 0.1 kg (WBSF) depending on use of the model for either Select or upper two-thirds Choice.

Freezing and retail display reduced the frequency of steaks failing to be certified as tender (Table 3.3). Probability of tough steaks based on WBSF ≥ 4.4 kg was unable to be analyzed as one treatment had no observed tough samples. Slice shear force data showed a numeric decrease across all treatments for probability of less than tender samples (SSF ≥ 20.0 kg). Generally speaking, the sample population in Trial 1 was relatively tender. Warner-Bratzler shear force and SSF measurements may have plateaued on the extreme low end, making differentiation of the effect of simulated shipping on shear force more challenging. The results of Trial 1 indicated trends for reduced shear force values as a result of increased sample handling. Trial 2 was conducted to evaluate the effect of actual shipping conditions on shear force values.

Trial Two – Non-Simulated Shipping Conditions

Temperatures recorded inside coolers during shipment in Trial 2 found a range in temperatures between coolers to be ± 6.7° C. This observations represents the potential for tenderness survey samples to be exposed to conditions that are likely deleterious to accurately

reflecting tenderness at the retail level. Data from Trial 2 displayed similar trends to those in Trial 1 (Table 3.3). The reduction in mean shear force values of samples sheared fresh compared to those sheared following freezing, and those exposed to retail display were nearly identical between Trials 1 and 2. Between samples sheared fresh and those exposed to shipping, the frequency of tough samples within the sample population was reduced from an initial incidence of over 30% to around 5% and over 36% to nearly 28% as determined by WBSF and SSF, respectively (Table 3.3). Probability of tough samples based on WBSF was also nearly zero following shipping.

Conclusions

Tenderness surveys are valuable tools that allow the beef industry to monitor sensory attributes of beef at the retail level. However, results of this work indicate that surveys are likely heavily influenced by the protocols used during sample collection and shipment. Without consideration of the influence of sample shipping, handling and shear force protocol, the conclusions drawn from these works may be less accurate and precise. Shipping samples frozen likely more accurately reflects tenderness at the retail level as samples shipped fresh and then frozen appear to be artificially tenderized as a result of shipping conditions. If tenderness surveys are to be relied upon to monitor progress and improve beef consumer demand, standardization of protocol is necessary to allow for comparison between studies. This protocol should dictate shipment of samples in a frozen state. Currently, NABTS data are in closest agreement with those from the early 1990's. This could demonstrate lack of progress by the industry in the area of beef tenderness, or a fundamental shift in factors influencing this trait. The current work indicated that 15 to 30% of top loin and sirloin samples are unacceptable in

terms of tenderness. The beef industry must increase efforts to improve tenderness in order to compete with other, less expensive, more consistent protein sources.

Table 3.1. Summary of sample population means for Warner-Bratzler shear force (WBSF) of top loin and sirloin samples collected at retail during major tenderness surveys.

	Top Loin Steak WBSF (kg)		Sirloin Steak WBSF (kg)	
Study	Mean	≥ 3.9[a] (%)	Mean	≥ 3.9[a] (%)
Morgan et al., 1991	3.25	4.0 – 21.0[b]	3.56	4.0 – 21.0[b]
George et al., 1999	1.91 – 3.19[b]	13.3	2.72 – 3.54[b]	20.5
Brooks et al., 2000	2.77	6.6	3.04	11.0
Voges et al., 2007	2.12	0.0	2.50	0.0
Savell, 2012	2.36	4.3	2.45	2.2
NABTS – Period 1[c]	2.87	15.0	3.54	29.2
NABTS – Period 2[c]	3.39	24.6	4.00	53.5

[a] WBSF ≥ 3.9 indicates samples predicted to be intermediate or tough in terms of tenderness (Platter et al., 2005).

[b] Data separated by quality grade. Range represents inclusion of all grades analyzed.

[c] North American Beef Tenderness Survey – Period 1: June 2011 to Nov 2011, samples shipped fresh, frozen on arrival; Period 2: Dec 2011 to May 2012, samples shipped frozen, stored frozen.

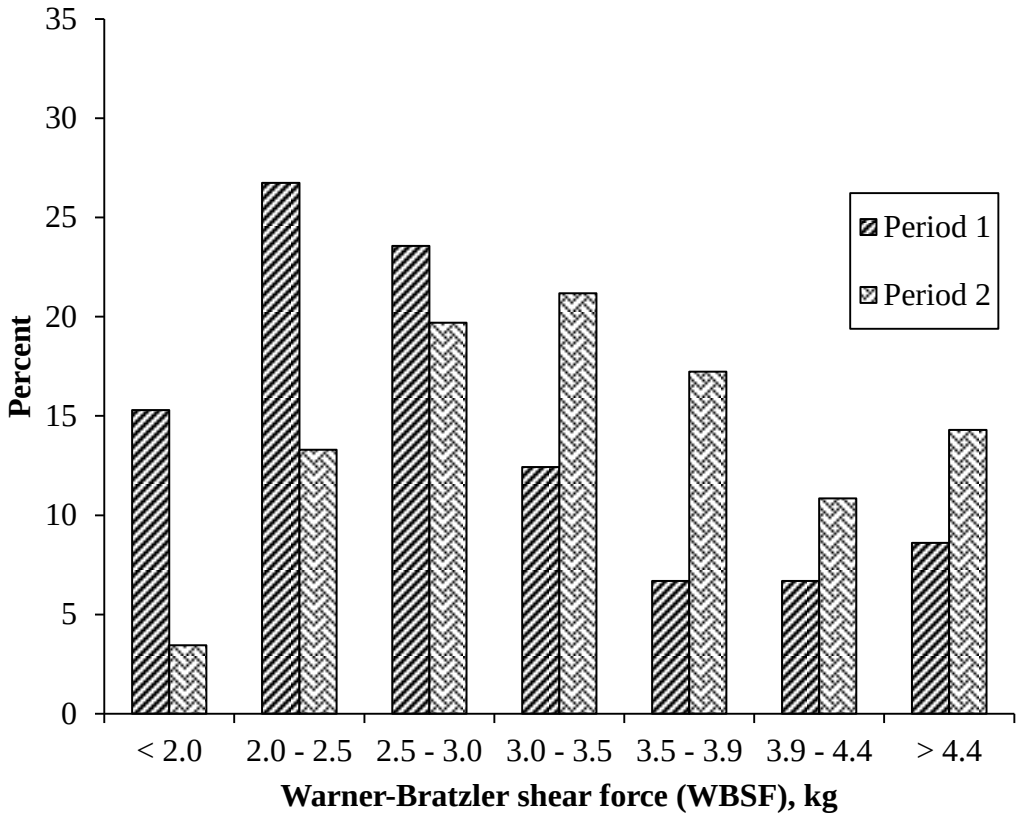

Figure 3.1. Frequency distribution of shear force values for top loin steaks collected as a part of the North American Beef Tenderness Survey. Period 1 – samples shipped fresh, frozen on arrival; Period 2 – samples shipped frozen, stored frozen.

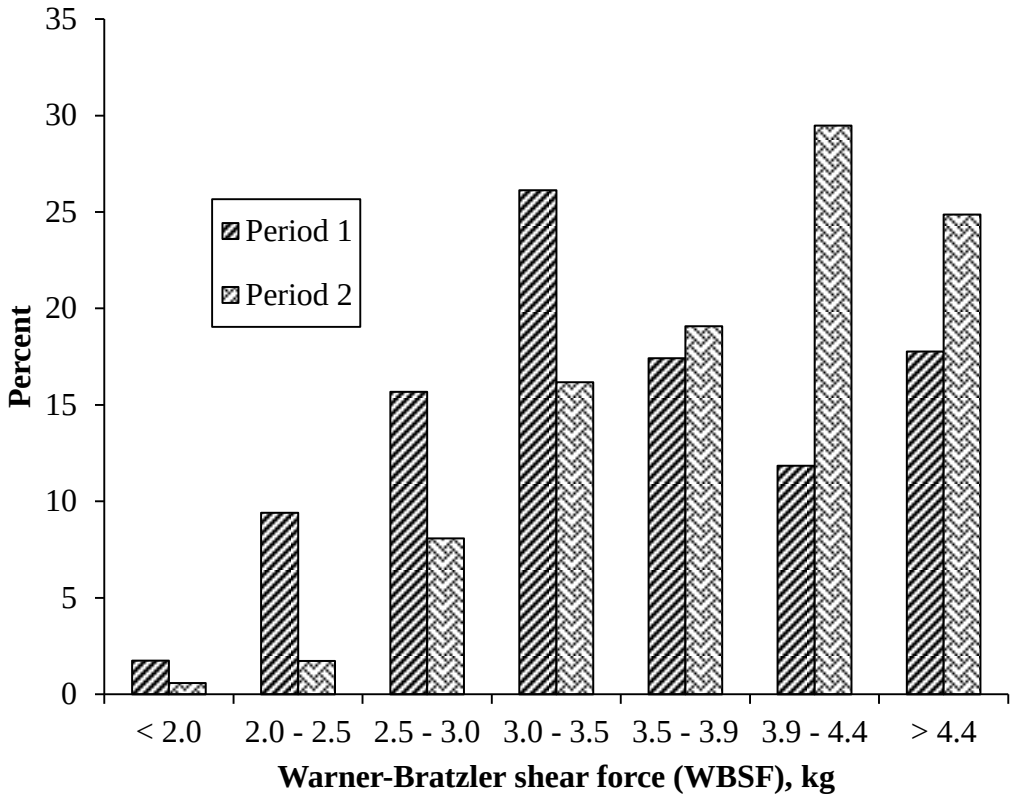

Figure 3.2. Frequency distribution of shear force values for sirloin steaks collected as a part of the North American Beef Tenderness Survey. Period 1 – samples shipped fresh, frozen on arrival; Period 2 – samples shipped frozen, stored frozen.

Table 3.2. Means and standard deviation ( ) for trained sensory panel ratings for beef top loin and sirloin steaks collected at retail locations across the U.S.

			Sensory Panel Ratings[a]				
Period	Cut	N	Overall Tenderness	Myofibrillar Tenderness	Connective Tissue	Juiciness	Beef Flavor
One[b]	Top Loin	272	9.3 (1.9)	9.4 (2.0)	9.4 (2.0)	7.6 (1.5)	8.5 (1.3)
	Sirloin	230	8.2 (1.9)	8.4 (1.8)	8.2 (1.9)	7.5 (1.6)	7.8 (1.2)
Two[c]	Top Loin	191	9.3 (1.6)	9.4 (1.5)	9.4 (1.7)	7.5 (1.3)	7.6 (1.3)
	Sirloin	169	8.6 (1.6)	8.9 (1.5)	8.6 (1.7)	7.5 (1.3)	7.1 (1.1)

[a] Ratings generated on a 15 cm line scale with 0 = extremely undesirable, 15 = extremely desirable for each attribute

[b] June 2011 – November 2011. Samples shipped fresh, frozen on arrival. Trained panel comprised of individuals with approximately 3-5 years of sensory panel experience. Regression analysis determined that panel rating equal to Warner-Bratzler shear force of 4.4 kg was 7.5 cm.

[c] December 2011 – May 2012. Samples shipped frozen, stored forzen. Regression analysis determined that panel rating equal to Warner-Bratzler shear force of 4.4 kg was 8.1 cm

Table 3.3. Warner-Bratzler shear force (WBSF), slice shear force (SSF), frequency and probability [P] of low Choice top loin steaks failing to be certified as tender (WBSF ≥ 4.4 kg, SSF ≥ 20 kg; ASTM, 2011) after different sample handling protocols.

		Treatment[a]					
Trial	Trait	Shear Fresh	Freeze/Shear	Display/Shear	Shipped	SEM	P_{TRT}
1	WBSF, kg	3.6[b]	3.2[c]	2.9[d]	2.9[d]	0.2	<0.0001
	SSF, kg	16.2[b]	16.0[b]	15.9[b]	14.7[c]	0.9	0.0480
	WBSF ≥ 4.4 kg, %	20.4	11.1	0.0	3.8		
	SSF ≥ 20 kg, %	19.4	13.9	11.1	5.7		
	[P] WBSF ≥ 4.4 kg[e]						
	[P] SSF ≥ 20 kg	0.14 (0.08)	0.09 (0.06)	0.06 (0.05)	0.03 (0.02)	*	0.2757
2	WBSF, kg	4.2[b]	3.8[c]	3.5[d]	3.4[d]	0.1	<0.0001
	SSF, kg	19.2	18.5	18.6	18.3	0.5	0.4594
	WBSF ≥ 4.4 kg, %	31.5	22.2	13.0	5.6		
	SSF ≥ 20 kg, %	36.1	36.1	30.5	28.6		
	[P] WBSF ≥ 4.4 kg	0.23[b] (0.20)	0.12[bc] (0.12)	0.05[cd] (0.06)	0.02[d] (0.02)	*	0.0012
	[P] SSF ≥ 20 kg	0.34 (0.13)	0.34 (0.13)	0.28 (0.11)	0.27 (0.11)	*	0.8698

[a] Steaks aged 14 d, then sheared fresh, overwrapped and frozen then sheared (Freeze/Shear), overwrapped displayed 68 h in retail case then sheared (Display/Shear) or displayed 68 h and exposed to shipping conditions. Trial 1 – simulated shipping, samples placed on cargo truck for 24 h. Trial 2 – samples shipped from Fort Collins, CO to Stillwater, OK via overnight air delivery.

[b-d] Means within a row lacking a common superscript differ ($P < 0.05$).

[e] Estimates could not be separated due to frequency of zero within Display/Shear treatment.

* Standard error for each estimate reported in parenthesis.

CHAPTER IV

EFFECT OF BETA-AGONIST SUPPLEMENTATION ON LIVE PERFORMANCE, CARCASS CHARACTERISTICS, FABRICATION YIELDS AND BEEF QUALITY OF CALF-FED HOLSTEIN STEERS

INTRODUCTION

Dairy-type carcasses have been estimated to comprise 9.9% of those presented for grading at fed-beef facilities (Moore et al., 2012). Carcasses exhibiting dairy type have traditionally been discounted, despite deposition of greater marbling amounts (McKenna et al., 2002; Moore et al., 2012) and retail yield (Luzardo, Unpublished). Discounts reflect low consumer acceptance of size and shape of top loin steaks from dairy type cattle. Beta-agonists may be a mechanism to modify size and shape of steaks from dairy type cattle (Lawrence et al., 2011), while increasing antemortem performance and carcass weight (Bass et al., 2009; Beckett et al., 2009; Boler et al., 2009; Garmyn et al., 2010; Haneklaus et al., 2011).

Beta-agonists have been reported to increase hot carcass weight (HCW) and ribeye area (REA), and reduce marbling score and tenderness in beef breeds (Dikeman, 2007). Consequences of use may be dependent on compound and supplementation level (Avendaño-Reyes et al., 2006; Scramlin et al., 2010; Arp, 2012). Negative effects of beta-agonists on quality could be less noticeable in higher quality cattle, such as calf-fed Holstein steers. Conflicting research exists with respect to the ability of consumers to detect differences in sensory attributes of steaks from Holstein steers fed zilpaterol hydrochloride (ZH) (Hilton et al., 2009; Mehaffey et al., 2009; Garmyn et al., 2010). A number of works have examined effects of ZH on calf-fed Holsteins (Beckett et al., 2009; Boler et al., 2009; Holmer et al., 2009; Mehaffey

et al., 2009; Garmyn et al., 2010; Hameklaus et al., 2011), while a limited amount of literature exists on the effects of ractopamine hydrochloride (RH) in calf-fed Holstein steers (Bass et al., 2009; Vogel et al., 2009). It is necessary to quantify the effect of each compound to optimize management. The objective of this study was to determine the effect of both RH and ZH on live performance, carcass characteristics, subprimal yield and eating quality of calf-fed Holstein steers.

MATERIALS AND METHODS

Steers were finished in southern AZ during November and December and harvested at a nearby commercial facility. On arrival, steers were implanted with a progesterone (100 mg) plus estradiol benzoate (10 mg) combination implant (Synovex®-C), vaccinated with an 8-way clostridial (Covexin®-8), IBR/PI_3 (Nasalgen®-IP) and IBR/BVD/PI_3/Leptospirosis (Titanium®-4L5) vaccine. Additionally, all steers were treated with the antibiotic florfenicol (Nuflor®) and received ivermectin pour-on (Noromectin). Steers were re-implanted with a terminal trenbolone acetate (200 mg)/estradiol (40 mg) (Revalor®-XS, Merck Animal Health, Summit, NJ) implant and blocked by weight into pens (N=32) consisting of ninety steers each. Eight blocks were present in the study with each treatment represented once within each block. Pens within blocks were randomly assigned to one of four treatments: no beta-agonist (control), RH at 300 or 400 mg/hd/d fed for 30 d prior to harvest or ZH (6.8 g/ton) fed for 23 d prior to harvest, with a 3 d withdrawal. Steers were finished on the diet outlined in Table 4.1. Data for live performance among control and beta-agonist treatments were collected via feedlot closeout data. Steers were harvested over four weeks with two pens/treatment (two blocks) represented each week.

Carcasses from implant and beta-agonist treatments were chilled for 48 h before grading. Grade data were collected online using a portable VBG 2000 VIA system (e+v Technology GmbH & Co.KG, Oranienburg, Germany). Eight to nine sides per pen were randomly selected for cutout assessment. Fourteen carcasses per pen were randomly selected for SSF determination. Samples approximately 6.35 cm thick were excised from the anterior portion of the strip loin from both sides of carcasses selected for SSF determination. One sample from each side was randomly assigned to either 14 or 21 d aging periods. During harvest weeks 2 and 3, eighty USDA low Choice sides within those selected for cutout were identified for purposes of collection of subprimal cuts for retail yield cutout and collection of steaks for trained sensory panel evaluation. Retail cutout and sensory panel evaluation included all treatments except RH fed at 400 mg/hd/d.

Carcass Cutout

Cutout data were collected within a commercial facility using plant personnel to fabricate sides into items typically merchandised by the facility (Appendix A). Sides were fabricated at a rate of one side every 3 to 4 minutes in ascending order of quality grade with no randomization of lot within quality grade. Chilled side weight was collected upon entry on to the fabrication floor; primal weights were obtained for each carcass side; subprimal, trim, fat and bone weights were summed back to chilled side weight with an acceptability range for weigh back (i.e., theh sum of all of the parts in relation to the initial weight) of ± 2.0%. This sensitivity allowed use of 342 carcass sides within the dataset. Trim was evaluated for percent fat using a MeatMaster™ (FOSS, Hilleroed, Denmark). Trim samples were evaluated by the MeatMaster™ in plastic bags that contained trim components from each carcass side. Samples for SSF and trained sensory panel determination were transported either fresh or frozen on dry ice from southern AZ to

Colorado State University. Samples were evaluated for temperature upon arrival; those that were inadvertently frozen during transport were removed from the sample population. Remaining samples were either placed into storage at -28.8° C, or at 4° C to complete the specified postmortem aging time.

Slice Shear Force Determination

Following freezing, samples for SSF determination that were excised from carcass sides as 6.35 cm thick portions of loin were fabricated on a band saw to a thickness of 2.54 cm with external fat trimmed to approximately 0.3 to 0.6 cm. Steaks remained frozen for at least one wk prior to SSF determination. Steaks were tempered for 24 h at 4° C to a target pre-cooking internal temperature of 1 to 3° C. Cooking was completed using a GRP99 Next Generation Grill (Spectrum Brands Inc., Madison, WI). Steaks were cooked to a target peak internal temperature of 71.1° C. Slice shear force (SSF) was conducted using the methodology described by Shackelford et al. (1999) on an Instron Universal Testing Machine (Model 4443, Instron Corporation, Canton, MA) using a cross-head speed of 500 mm/min.

Trained Sensory Panel

Panelists (n = 10) were trained for evaluation of nine sensory attributes including myofibrillar tenderness, connective tissue tenderness, overall tenderness, juiciness, beef flavor, buttery, metallic, livery, and grassy. Training was conducted using beef *Longissimus dorsi, Biceps femoris, Gluteus medius, Infraspinatus, Semitendinosus* and *Psoas major.* Flavor profile training was conducted using the standards published by Adhikari et al. (2011). Steaks 2.54-cm thick were fabricated from low Choice strip loins using a scalloping blade fitted to a commercial band saw. Two of the first eight steaks fabricated from the anterior portion were retained and aged 14 or 21 d postmortem before freezing. All samples remained frozen for at least one wk

before trained panel evaluation. Thawing and cooking were conducted in a manner identical to that described for SSF determination. Samples were trimmed of external edges and connective tissue before being portioned into cubes (1.27 x 1.27 x 2.54 cm). Panelists were served two cubes under incandescent red-lighting and provided with unsalted crackers, apple juice and distilled water to cleanse the palate. Responses were marked on a 15 cm continuous line scale (Appendix B).

Statistical Methods

Plots of residuals and the W-statistic (Shapiro and Wilk, 1965) were evaluated to determine homogeneity of variance for all data. Denominator degrees of freedom were calculated using the Kenward-Roger approximation (Kenward and Roger, 1997) and means were separated using pairwise t-tests and a significance level of 0.05. SAS 9.3 (SAS Institute, 2013) was used for all data analysis. Mixed models were analyzed using the MIXED procedure. The GLIMMIX and GENMOD procedures were used to analyze frequency data. By default, these procedures are identical if the random effect statement is removed from the GLIMMIX procedure. Analysis using the GENMOD procedure was equivalent to a chi-squared test for frequency data.

Pen level data for feedlot performance and carcass cutout were analyzed as a randomized complete block design, with a random effect that grouped blocks by fabrication or harvest day. Individual carcass level data, e.g. carcass characteristics and shear force, were analyzed using a mixed model that included random block and fabrication day effects. Models included a random treatment by block interaction to separate an appropriate pen level error term for testing treatment effects. Additionally, models for individual data included covariates for peak internal cook temperature (degree of doneness) and marbling score initially, but covariates were removed

due to lack of significance ($P > 0.05$). It should be noted that marbling score does not meet the classical definition of a covariate as it could be influenced by treatment, however treatment was not influential to marbling in this sample population ($P > 0.05$), nor was marbling significant as a covariate for shear force. The treatment by block term was identical to the effect of lot or pen since only one replicate was represented per block. Sensory panel data were evaluated using a mixed model that included fixed effect of treatment and a random effect of panel. Sensory panel samples were collected from only low Choice carcasses within control, RH 300 and ZH treatments during weeks two and three. Consequently, panel data by block was highly unbalanced and the effect of block was not used in models for sensory panel responses.

Individual carcass level data for quality and yield grade classification were analyzed using generalized linear mixed models in the SAS procedure GLIMMIX (SAS Institute, 2013) with the same terms and methodology described above for models that evaluated individual level data e.g. carcass characteristics, shear force. Proportions of carcasses classified as No Roll, Select, Choice or Prime and YG 1-5 were analyzed separately in binomial models with a logit link. Frequency data for the probability of a steak failing to be certified as tender were evaluated using simplified generalized linear models in the SAS procedure GENMOD (SAS Institute, 2013). This simplification of the model statement was conducted following evaluation for over dispersion of the data (variance > μ) and determination that variance attributed to the block effect was zero. The Pearson chi-squared statistic divided by degrees of freedom showed no over dispersion. Full description of methods used to determine the appropriate model for tests of frequency of tough vs. tender steaks is presented in Appendix D.

RESULTS AND DISCUSSION

Feedlot Performance

Least squares means for feedlot performance calculated by lot from closeout data are presented in Table 4.2. Treatment did not affect initial or final BW, which was in agreement with the findings of Bass et al. (2009), Beckett et al. (2009), but different from those of Avendaño-Reyes et al. (2006), Vogel et al. (2009) and Scramlin et al. (2010) who found increased final weights in cattle treated with RH and ZH. Beta-agonists improved feed efficiency (FE) 18 to 25% ($P < 0.05$). Bass et al. (2009) and Beckett et al. (2009) found more modest improvements in FE of calf-fed Holstein steers (< 5%), but our findings were nearly identical to those of Vogel et al. (2009) who evaluated RH administered to calf-fed Holsteins, and to those of Scramlin et al. (2010) and Arp (2012) who evaluated RH and ZH in populations comprised of beef breeds. This current work found that beta-agonists reduced DMI 4 to 7% and increased ADG 15 to 16% ($P < 0.05$). The decrease in DMI was greater than in any previously published research that has evaluated both RH and ZH within the same sample population, or either beta-agonist in populations of calf-fed Holstein steers. Improvements in ADG were most similar to the finding of Vogel et al. (2009).

Carcass Composition

Data summarizing effects of beta-agonists on carcass traits are presented in Table 4.3. Beta-agonists affected most carcass traits evaluated, but no significant differences between RH 300 and 400 treatments were observed, except for a higher rate of total liver abscess in the RH 300 treatment; reasons for this increase were unknown. Beta-agonists increased HCW, dressing percentage and *Longissimus* muscle area (LMA). Zilpaterol hydrochloride had a more dramatic effect on these traits than did RH ($P < 0.05$). Twelfth rib fat (FT) was slightly reduced due to

beta-agonists ($P < 0.05$), but the sample population was generally very lean with average FT less than 0.8 cm. Marbling score and percent kidney, pelvic and heart fat were not affected by beta-agonist treatment.

Beta-agonists increased dressing percentage by 0.6 to 1.8% and HCW by 2 to 4% ($P < 0.05$). These findings were in agreement with most previous works (Bass et al., 2009; Beckett et al., 2009; Vogel et al., 2009; Scramlin et al., 2009; Arp, 2012); however, Avendaño-Reyes et al. (2006) reported increases of over 4% in HCW following administration of RH and ZH to a population of Mexican-bred steers. The current work found greater increases in HCW and dressing percent (DP) in cattle fed RH than most previous reports, except for that of Avendaño-Reyes et al. (2006). The current work found greater increases in HCW and DP than those reported by Beckett el al. (2009) who investigated effects of ZH on calf-fed Holstein steers. Zilpaterol hydrochloride increased the percentage of carcasses weighing over 430 kg ($P < 0.05$). However, only four carcasses in the sample population weighed over 476 kg, three from the ZH treatment and 1 from the RH 400 treatment. Distribution of HCW by treatment showed increased frequencies of carcasses below 362 kg in the control treatment and over 408 kg in the zilpaterol treatment (Figure 4.1).

Use of beta-agonists increased LM area compared to controls ($P < 0.05$). The shift in distribution of LM area appeared to occur above and below 13.0 in^2 (Figure 4.2). The increase in LM area exceeded that of HCW on a percent basis, which should decrease yield grade. Ractopamine hydrochloride increased LM area by 3.6 to 3.8%, which is in relative agreement with other works that have evaluated the effect of RH on calf-fed Holsteins (Bass et al., 2009; Vogel et al., 2009). Zilpaterol hydrochloride increased LM area by 9.7%, which was similar to the changes observed by Avendaño-Reyes et al. (2006), Scramlin et al. (2010) and Beckett et al.

(2009), but greater than those reported by Arp (2012). Most research has shown a 5 to 10% increase in LM area of cattle fed ZH, while improvements in LM area following use of RH have been reported to be 2 to 7%. The smaller response to beta-agonists in beef breeds compared to calf-fed Holsteins and Mexican-bred steers could indicate potential for greater muscle hypertrophy in inherently lighter muscled populations, possibly due to differences in fiber type or diameter.

Reduced subcutaneous fat was observed as a result of beta-agonist supplementation ($P < 0.05$); however beta-agonists had no effect on perinephric fat ($P > 0.05$). Compared to controls, ZH decreased twelfth rib fat (FT) by 8.7% and RH reduced FT approximately 5% ($P < 0.05$). Previous studies have reported reductions in twelfth rib fat (FT) of 0 to 17% following treatment with either RH or ZH (Avendaño-Reyes et al., 2006; Bass et al., 2009; Beckett et al., 2009; Vogel et al., 2009; Scramlin et al., 2009; Arp, 2012). Despite statistical significance between treatments, changes in FT on an absolute basis were less than 0.1 cm which was indicative of a sample population that was inherently very lean. This could explain the findings of Beckett et al. (2009) who reported no difference in FT for steers fed ZH for 20 d compared to controls. Kidney, pelvic and heart fat was not different in steers treated with beta-agonists ($P > 0.05$). Numerically, the observed difference in KPH was greater than any previous research that has evaluated the influence of beta-agonists in calf-fed Holsteins, or both RH and ZH within the same sample population comprised of beef breeds.

Numeric Yield Grade (YG) was lower in carcasses from steers fed beta-agonists ($P < 0.05$), but the range in least squares means for calculated YG was only 0.3. Ractopamine hydrochloride improved YG by approximately 0.1 compared to controls, while ZH improved YG by 0.2 compared to controls ($P < 0.05$) (Table 4.3). Approximately 95% of carcasses calculated

to be either a YG 1 or 2 (Table 4.4); thus were very lean on average. Use of beta-agonists increased percentage of YG 1 carcasses ($P < 0.05$) and reduced YG 2 carcasses. Very few YG 3 and no YG 4 carcasses were observed in the sample population. Zilpaterol hydrochloride increased the percentage of YG 1 carcasses by over 30% compared to controls, and nearly 20% compared to RH 400 ($P < 0.05$). However, findings related to calculated YG may not be completely indicative of differences in subprimal yield. Lawrence et al. (2010) reported that the USDA Yield Grade equation was not accurate in expressing differences in subprimal yield from carcasses of calf-fed Holstein steers. Our findings substantiate this conclusion in that final YG, as determined by VIA systems, explained less than half ($R^2 = 0.47$) of the observed differences in subprimal yield. Specific to this dataset, KPH alone was able to explain 48% of the variation in subprimal yield, a logical finding when considering the nearly linear reduction in KPH resulting from treatment with beta-agonists. These findings indicated that further research may be necessary to determine algorithms more capable of predicting subprimal yield of carcasses from calf-fed Holstein steers, particularly those treated with beta-agonists.

Carcass Quality

Beta-agonists have been summarized to negatively impact beef quality (Dikeman, 2007; Delmore et al., 2010). The current work found that steers receiving beta-agonists had no difference in marbling score compared calf-fed Holstein steers administered a terminal TBA/estradiol combination implant ($P > 0.05$) (Table 4.3). This contradicted the findings of many studies that evaluated effects of beta-agonists on populations comprise of beef breeds; however, Bass et al. (2009) reported no difference in marbling scores between calf-fed Holstein steers supplemented with RH and controls. Most other studies have found RH to reduce marbling by 10 to 20 degrees, while ZH has been reported to reduce marbling by over 20 degrees

(Avendaño-Reyes et al., 2006; Beckett et al., 200; Vogel et al., 2009; Scramlin et al., 2010; Arp, 2012). Cattle managed under the natural protocol in the present study demonstrated high potential for calf-fed Holstein steers to deposit intramuscular fat (Appendix C). This genetic potential, coupled with increased days on feed, optimized marbling development. A cattle population that has a higher genetic potential for deposition of intramuscular fat and one that is fed for longer periods of time may lend itself more readily to use of more aggressive growth promotants without noticeable effects on meat quality. This hypothesis would agree with the findings of Rathmann et al. (2012) who reported that beef heifers supplemented with ZH and placed on feed for extended periods had comparable quality grade and marbling scores to non-supplemented controls fed for shorter periods.

The percentage of steers that graded low Choice was comparable between all treatments ($P > 0.05$) (Table 4.4). Controls had a greater frequency of carcasses that graded in the upper two-thirds of Choice compared to beta-agonist treatments ($P < 0.05$). Frequency of carcasses that graded Select was 6% higher in cattle supplemented with ZH relative to controls ($P < 0.05$). Distribution of marbling scores by treatment demonstrated minimal shifts in marbling score between treatments (Figure 4.2). These findings agreed with those of Arp (2012) who reported no influence of beta-agonists on the percentage of carcasses that graded low Choice, but, instead, a reduced frequency of carcasses grading in the upper two-thirds of Choice due to treatment with beta-agonists. These influences could be extremely significant to branded beef programs sourcing beef products from carcasses graded in the upper two-thirds of Choice.

Carcass Cutout

To preface, works that have evaluated subprimal cutout yields of cattle fed beta-agonists have typically investigated only one of the two commercially available compounds (Boler et al.,

2009; Holmer et al., 2009; Kellermeier et al., 2009; Rathmann et al., 2009; Garmyn et al., 2010; Hilton et al. 2010), applied these compounds to only populations comprised of beef breeds (Arp, 2012), or used carcass sampling procedures that were not totally random. This study is the only work that has evaluated both commercially available beta-agonists in calf-fed Holsteins, determined subprimal yield based on data generated within commercial facilities, and done so using selection criteria that were totally random.

Compared to controls, beta-agonists increased saleable yield of whole-muscle cuts by 0.61%, 0.86% and 1.95% for RH 300, RH 400 and ZH, respectively ($P < 0.05$) (Table 4.5). Percent fat was lower in carcasses from the ZH treatment compared to controls ($P < 0.05$); however, this difference was not observed between RH treatments and controls ($P > 0.05$). Percent bone was lower in the ZH treatment due to increased muscle ($P < 0.05$). The percent of chilled side weight comprised of trimmings was unchanged between treatments, but on a 100% lean basis, RH 400 and ZH increased trim yields ($P < 0.05$) (Table 4.6). Analysis of saleable yield by primal showed a fundamental shift in growth and development. Beta-agonists caused a shift in proportion of saleable yield within individual primals, with a greater portion produced from the hindquarter relative to the forequarter ($P < 0.05$) (Figure 4.4).

Scientific literature regarding growth and development of bovine animals reveals that although changes in animal size and weight may occur (such as those following administration of hormone based implants), the ratio of one muscle group to another normally remains relatively constant within species (Berg and Butterfield, 1968; 1976). The present study suggests that beta-agonists caused a shift in muscle growth and development patterns, increasing the proportion of muscles of the hindquarter relative to those of the forequarter, specifically affecting muscles of the round (Figure 4.5). This could be due to increased sensitivity of type II muscle fibers to beta-

agonists (Smith et al., 1995) and the relatively high content of white muscle fibers in muscles of the hindquarter (Kirchoffer et al., 2002). However, the changes in development observed in the present study were not reflected in data presented by Arp (2012) who explored the same growth promotants in a population of comprised of beef breeds (Figure 4.6).

The different response to beta-agonists based on breed type could be due to muscle fiber demographics; however published research has reported conflicting evidence to support this hypothesis. Spindler et al. (1980) reported that smaller diameters of white muscle fibers were present in Holstein steers compared to Angus and Hereford cattle. The same study also reported relatively unchanged percentages of white muscle fibers between breeds (Spindler et al., 1980). If the white fibers present in Holstein steers are indeed smaller, they could lend themselves more readily to muscle hypertrophy. However, if percentage is more important, these findings may not support increased response of Holstein steers to beta-agonists. Dreyer et al. (1977) reported an increased percentage of type II fibers in Holstein cattle compared to Afrikaner cattle, a *Bos indicus* breed that may not be comparable to breeds traditionally used in the U.S. for beef production. Other studies have reported that an increased plane of nutrition can result in a greater percentage of type II muscle fibers (Siedeman and Crouse, 1986). Management of calf-fed Holstein steers dictates higher planes of nutrition throughout life compared to those of beef breeds. This, coupled with the potential for increased percentages of white muscle fibers, could explain an increased sensitivity to beta-agonists. If calf-fed Holstein steers are indeed more sensitive to beta-agonists, this could result in added return on invest for producers supplementing Holstein steers with beta-agonists.

Changes in development were reflected by an increased percentage of chilled side weight comprised of muscles from the round when calf-fed Holstein steers were treated with beta-

agonists ($P < 0.05$) (Table 4.7). Top round, bottom round, eye of round, heel and knuckle subprimals all comprised a greater percentage of chilled side weight in RH 400 and ZH treatments than controls ($P < 0.05$), and top round, bottom round, eye of round and heel comprised a greater percent of chilled side weight in ZH treated cattle than in any other treatment ($P < 0.05$). Previous research has reported nearly identical findings in Holstein steers and beef breeds supplemented with ZH and/or RH (Boler et al., 2009; Hilton et al., 2009; Kellermeier et al., 2009; Rathmann et al., 2009; Garmyn et al., 2010; Hilton et al. 2010; Scramlin et al., 2010; Arp, 2012). The current findings are in relative agreement with those studies in that one of the only cuts evaluated in the loin, the loin flap or *Obliquus abdominis interni*, was not affected by beta-agonists. This finding was interesting considering that Berg and Butterfield (1976) reported the *Obliquus abdominis interni* to be a high impetus muscle, which the authors summarized to be used more heavily as weight increases. If weight is more influential to this muscle than hormone based mediators of growth, this could explain the lack of response. Interestingly, no effect of beta-agonists on the percent of chilled side weight comprised of the strip loin was observed ($P > 0.05$). Due to the shallow shape of the *Longissimus dorsi* in medial sections of the strip loin of calf-fed Holstein steers, excess subcutaneous fat may have been included in strip loins from certain treatments, which could have negated the effect of beta-agonists on this cut. Carcasses from steers treated as controls or with RH did not differ in percent chilled side weight comprised of tenderloin ($P > 0.05$); however, tri-tip and top sirloin butt accounted for a greater percentage of chilled side weight in RAC 400 compared to controls ($P < 0.05$). Most loin cuts (except strip loin and loin flap) from carcasses of steers treated with ZH comprised a greater percentage of chilled side weight compared to all other treatments ($P < 0.05$). Relative to trim components, fat from the loin and round was lower in carcasses of steers provided beta-agonists, but not different

between RH treatments ($P > 0.05$). Zilpaterol hydrochloride reduced percent bone in the round, but not in the loin. Percent trim from the round was higher carcasses of steers treated with beta-agonist compared to controls ($P < 0.05$). To summarize, increased dose or potency of beta-agonists caused an increased percentage of chilled side weight to be present in the form of cuts from the hindquarter. The differential response of individual muscles to beta-agonists is without doubt economically significant to the packing industry.

Effects of beta-agonists on the cuts of the forequarter were less pronounced. This was likely caused in-part by fabrication styles that allowed for inclusion of seam fat depots within several cuts of the chuck and rib in carcasses of non-treated steers. These depots may have artificially increased cut weight, despite advantages in lean value found in cuts derived from carcasses of steers treated with beta-agonists. The shoulder clod (*Triceps brachii* and *Infraspinatus*), *Teres major* and *Supraspinatus* generally comprised a greater percentage of chilled side in RH and ZH treatments ($P < 0.05$). These effects were neither linear, nor uniform as dose and potency of beta-agonist increased, possibly indicating reduced sensitivity of these muscles to beta-agonists. The dorsal portion of the deep pectoral muscle that remained attached to the under blade comprised a greater percentage of chilled side weight for carcasses of steers in the ZH treatment, although no difference existed between carcasses of steers in the control and RH treatments. The ribeye roll and *Latissimus dorsi* comprised a greater percentage of chilled side weight for carcasses of steers in the ZH treatment ($P < 0.05$); however, no differences were observed between carcasses of steers treated with RH and controls in the same muscles. Fat and bone from the chuck were lower for carcasses of steers in the ZH treatment relative to controls ($P < 0.05$), however not different between carcasses of steers treated with RH and controls ($P > 0.05$). Previous works reported more mixed responses of cuts of the forequarter to ZH and RH.

Boler et al. (2009) reported no differences in percent chilled side weight comprised of cuts from the chuck following administration of ZH to calf-fed Holstein steers for 20 d. However, the same work found an increase in percent of chilled side weight comprised of the ribeye roll in carcasses from cattle fed ZH. Arp (2012) reported no effect of beta-agonists on percent of chilled side weight made up of any cut from the forequarter. When ZH was administered to beef steers, several workers found similar results to those presented herein (Hitlon et al., 2009; Kellermeier et al., 2009; Rathmann et al., 2009; Hilton et al., 2009; Garmyn et al., 2010; Scramlin et al., 2010).

The only thin meat (flank, inside and outside skirt) cut that increased in percent of total chilled side weight as a result of beta-agonists was the flank. Outside skirts were generally unchanged between treatments, however percent of chilled side weight comprised of inside skirt tended to be higher in carcasses of cattle fed ZH. Percent of chilled side weight comprised of inside skirt was higher in beta-agonist treated steers compared to controls ($P < 0.05$). The response of the inside skirt to ZH also was reported by Kellermeier et al. (2009), Rathmann et al. (2009), Hilton et al. (2009) and Garmyn et al. (2010). However, Boler et al. (2009) and Arp (2012) reported no effect of ZH on thin meat yields. Cuts merchandized from the plate, or navels, comprised a greater percentage of chilled side weight in controls ($P < 0.05$), likely due to increased fat content within this cut.

Effect on Shear Force

Dikeman (2007) concluded that beta-agonists increased shear force values of beef. Delmore et al. (2010) concluded that due to muscle hypertrophy caused by ZH, shear force values would be greater in steaks from cattle fed Zilmax®. Relative to the current work, RH and ZH increased slice shear force (SSF) and reduced probability of steaks meeting SSF

requirements to be certified tender (SSF < 20.0 kg; ASTM, 20011) (Table 4.7). Steaks had lower SSF values at 21 d postmortem than steaks aged to 14 d in all treatments ($P < 0.05$). At 14 d postmortem, the probability of a steak from the ZH treatment failing to be certified as tender was over 0.40, compared to just over 0.30 in both RH treatments; these probabilities were significantly higher than controls ($P < 0.05$), and there was a trend for steaks from carcasses of steers treated with ZH to have increased probability of failing to be certified as tender compared to steaks from carcasses in the RH 300 treatment ($P = 0.0708$). In steaks aged 21 d postmortem, the probability of failing to meet SSF requirements to be certified as tender was improved by 0.13 to 0.15 in all beta-agonist treatments. However, steaks derived from steers provided with the more aggressive treatments (RH 400 and ZH) still exceeded a probability of 0.20 of failing to be certified tender, which was greater than controls that were just over 0.10 ($P < 0.05$). Steaks from steers supplemented with the more moderate dose of RH (RH 300) had an incidence of steaks failing to be as certified tender that was more similar to steaks from steers in the control group, however steaks from steers treated with RH at 300 mg/hd/d did yield greater SSF values at 21 d postmortem compared to steaks from controls ($P < 0.05$). The response of SSF to increasing dose and potency of beta-agonists was nearly linear from controls to RH to ZH (Figure 4.5). Compared to controls, beta-agonists increased shear force by 12 to 25% at 14 d postmortem and 9 to 21% at 21 d postmortem ($P < 0.05$). Previous work has reported similar increases in Warner-Bratzler shear force (WBSF) of calf-fed Holstein steers fed ZH (Holmer et al., 2009; Garmyn et al., 2010). Mehaffey et al. (2009) reported more modest increases in SSF of calf-fed Holstein steers fed ZH. Few reports of the effect of RH on shear force of steaks from calf-fed Holstein steers have been published. Comparisons of RH and ZH within the same sample population of beef steers have found shear force to increase by 7 to 15% in RH

treatments compared to controls (Scramlin et al., 2010; Arp, 2012). The current work found a shift in distribution of slice shear force around approximately 20 kg at 14 d postmortem and around 16 kg at 21 d postmortem. A substantially increased incidence of slice shear force observations greater than 20 kg were observed in beta-agonist treatments at both aging periods (Figures 4.6 and 4.7).

Effects of beta-agonists on tenderness at differing quality grades have been examined in Holstein steers with varying conclusions. In top loin steaks from cattle fed ZH for 20 d, Mehaffey et al. (2009) reported that consumers were able to detect differences in tenderness in Choice steaks at 14 d postmortem, but not in Select steaks at the same age. By 21 d postmortem, the same authors reported no difference in tenderness of steaks from either quality grade (Mehaffey et al., 2010). Holmer et al. (2009) found that marbling did not influence shear force of steaks from cattle supplemented with and without ZH. The potential differences in tenderness at differing levels of marbling in steaks from calf-fed Holstein steers fed beta-agonists necessitated evaluation of SSF within various quality grades (Table 4.8 and 4.9).

Tests of hypotheses regarding differences in SSF means by Quality Grade at differing postmortem aging times were not an objective of the experiment. The sample size of the present work allowed these tests to be conducted with some power; however, not all mean separations represented in tables 4.8 and 4.9 are truly reflective of what might be found in a study that balanced observations by age and quality grade to determine differences in SSF. Probability data for SSF to exceed 20 kg as separated by Quality Grade (Table 4.8) were analyzed in a manner identical to that described above for the sample population at 14 and 21 d postmortem. Inclusion of block in models that analyzed data separated by age and quality grade was actually more inappropriate due to exceptionally limited sample size in certain blocks. This is relevant since

reduced observations in certain blocks led models that included the block term to predict artificially low or high frequencies for steaks with SSF > 20 kg. This effect artificially inflated the Pearson χ^2 statistic and resulted in failure to appropriately assess goodness-of-fit or over dispersion; consequently block was excluded from all models. Inclusion or exclusion of the block term in models had minimal effect on relationships between treatments. Footnotes in Table 4.8 summarize differences when block was included. Appendix D includes full model statements for separation of probability data.

Probability of steaks failing to meet SSF requirements to be certified as tender was higher in Select steaks from the ZH treatment at 21 d postmortem ($P < 0.05$). When data were evaluated by Quality Grade and aging period, sample size was reduced which negatively affected power. Consequently, the general effect of treatment on the frequency of a steak failing to be certified tender only approached significance in low Choice and Select samples at 14 d postmortem ($P \approx 0.12$). Within these categories, individual frequencies for samples to fail to be classified as tender were different between controls and ZH treatments ($P = 0.02$). Comparison of frequencies of failure to be certified tender between RH 300 and ZH approached significance ($P = 0.07$ and 0.11 for 14 d low Choice and Select samples, respectively). The RH 400 and ZH treatments tended to be different in failure to be certified tender in Select samples aged 14 d postmortem ($P = 0.11$). Tables 4.8 and 4.9 demonstrate that postmortem age may be more important than marbling to SSF of steaks from cattle fed beta-agonists, whereas marbling could be of greater importance to slice shear force of cattle managed without beta-agonists. To explore this effect, intra class correlation coefficients were calculated as $\sigma^2_{AGE} / \sigma^2_{AGE} + \sigma^2_{ERROR}$ from mixed models with the fixed effect of marbling and random effect of age used to predict SSF. Intra-class correlation coefficients indicated that postmortem age accounted for 2.9% of the

variation in SSF in steaks from the control treatment compared to 7.9% in steaks from beta-agonist treatments. In general linear models that used both age and marbling to predict SSF, the r^2 value for the beta-agonist treated cattle was only 0.05 compared to 0.63 for controls. Admittedly, these observations were influenced by a relatively low average SSF in the control treatment and the heavy influence of treatment on SSF.

The effect of beta-agonists on postmortem aging has been explored by workers who have found increased calpastatin and reduced calpain activity in meat from cattle fed beta-agonists (Wheeler and Koohmaraie, 1992). Reduced protein turnover could be partially responsible for increased muscle mass following beta-agonist use; however, this could negatively affect postmortem tenderization as a result of aging. Several researchers have failed to find differences in calpain or calpastatin activity of cattle fed ZH (Hilton et al., 2009), and most studies, including the current work, have found reduced shear force with increased days postmortem. However, if beef from cattle fed beta-agonists requires extended postmortem aging, shelf-life, flavor profile and purge losses could be negatively affected. Lastly, if marbling is a less suitable indicator of tenderness in cattle fed beta-agonists, current industry predictors of eating satisfaction could be impaired within cattle fed beta-agonists.

Trained Sensory Panel Evaluation

Trained panelists rated steaks from steers supplemented with ZH lower for overall tenderness compared to controls at 14 d and 21 d postmortem ($P < 0.05$) (Tables 4.10 and 4.11). Differences in overall tenderness between RH and ZH were non-significant at 14 d, but steaks from steers fed RH were more tender at 21 d postmortem ($P < 0.05$). Juiciness was lower in steaks from the ZH treatment at 21 d postmortem compared to controls and RH treatments ($P < 0.05$). Marbling score, as assessed by VIA imaging technology, was evaluated as a potential

covariate for beef flavor intensity and buttery/beef fat flavor. The range in least squares means for marbling score was 439 to 488, with a significant effect of treatment ($P < 0.05$). When used as a covariate, steaks from the RH treatment were rated higher for beef flavor at 14 d postmortem compared to steaks from the ZH treatment ($P < 0.05$). Marbling was not significant as a covariate for beef flavor at 21 d postmortem, but steaks from the RH and ZH treatments were rated lower for flavor intensity compared to controls ($P < 0.05$). Admittedly, use of marbling as a covariate is somewhat confounded in this instance since the effect of treatment was, at times, significant. However, differences in beef flavor intensity independent of marbling score are significant given the documented influence of marbling on trained panel ratings for flavor (Emerson et al., 2013). These findings may indicate that beta-agonists cause cellular differences in lipid content that could be influential to beef flavor. If lipid that is not visible is indeed influential, a new grading technology could be required to accurately predict differences in eating quality of cattle managed with beta-agonists.

The effect of RH and ZH on sensory panel ratings has been explored with mixed conclusions in the ability of consumer and trained panelists to detect differences in sensory attributes due to beta-agonists. Hilton et al. (2009) reported that trained panelists rated steaks from ZH treatments lower for all sensory attributes except the presence of off flavors ($P < 0.05$). This agreed with the current study where, across the entire sample population, steaks from the ZH treatment were rated lower for presence of livery off flavors ($P < 0.05$). Garmyn et al. (2010) found that trained panelists rated steaks from ZH treatments lower for sustained juiciness and overall tenderness ($P < 0.05$), in agreement with the current findings at 21 d postmortem. These findings were contrasted by consumer panel ratings where no differences were observed between steaks from control treatments and those from beef breeds fed ZH (Hilton et al., 2009).

Mehaffey et al. (2009) found similar results to those of Hilton et al. (2009) in steaks aged 21 d postmortem, but reported that consumers rated 14 d postmortem Choice steaks from Holstein steers fed ZH lower for tenderness, juiciness and overall like ($P < 0.05$). These findings were not observed in steaks from the same sample population that graded Select (Mehaffey et al., 2009). The current work showed that trained panelists were able to detect differences between control and ZH treatments in overall tenderness at 14 d and 21 d postmortem ($P < 0.05$), but results were not as decisive between RH and controls. Results of this study indicate that tenderness, juiciness and flavor may all be negatively impacted by beta-agonists, particularly zilpaterol hydrochloride.

Conclusions

This sample population showed that beta-agonists are highly effective tools to improve the growth, efficiency and subprimal yield of calf-fed Hosltein steers, likely having greater effects in populations of calf-fed Holstein steers compared to those comprised of beef breeds. These changes may not be fully quantified by Yield Grades used by the industry. This may be due to fundamental changes in development due to beta-agonist use, specifically increased proportions of subprimal yield from the round. Estimates of saleable yield from this work were comparable to some works that have evaluated non-beta-agonist supplemented populations of comprised of beef breeds, and indicated that beta-agonists could improve saleable yield of calf-fed Holstein steers to levels that are equivalent to certain groups comprised of beef breeds. Marbling was not affected by beta-agonist use, but this was not indicative of changes observed in shear force and trained sensory panel data. Beta-agonists increased 14 d postmortem shear force to levels that were well above the current industry status for incidence of samples failing to be certified as tender. This issue was magnified in cattle treated with zilpaterol hydrochloride that failed to meet the Choice grade. At 21 d postmortem, calf-fed Holstein steers treated with

ractopamine hydrochloride had comparable incidence of steaks failing to be certified as tender compared to that level documented at retail. This was untrue of steaks from steers treated with zilpaterol hydrochloride, where incidence was 10% and 22% greater at 21 d and 14 d postmortem, respectively. Within steaks from low Choice carcasses, zilpaterol hydrochloride reduced trained sensory panel ratings for overall tenderness, juiciness and flavor at 21 d postmortem. Differences in flavor profile were not accounted for by marbling and could indicate fundamental changes in cellular lipid content that are influential to beef flavor. This could reduce the capabilities of current industry predictors to determine eating satisfaction. Beta-agonists are an essential tool to improve efficiency, yield and productivity of the beef industry; however, they appeared in this study to be detrimental to beef quality. Quality attributes at retail must continually be monitored to determine the effect that beta-agonist use is having on consumer acceptance of beef products.

Table 4.1. Nutrient composition (DM basis) of the ration for calf-fed Holstein steers implanted with Synovex®-C and Revalor®-XS then finished with or without beta-agonists.

Ration Component	Content
Dry Matter, %	83.2
Crude Protein (CP), %	14.4
Non Protein Nitrogen, %	2.4
Forage Dry Matter	12.4
NDF, %	16.8
Calcium, %	0.7
Phosphorus, %	0.3
Monensin, mg/head/d	270.0

Table 4.2. Least squares means for feedlot performance of calf-fed Holstein steers implanted with Synovex®-C and Revalor®-XS and finished with or without supplementation in the diet with beta-agonists.

	Treatment[a]					
	Control	RAC 300	RAC 400	Zilpaterol	SEM	P_{TRT}
No. of Pens	8	8	8	8	-	-
Initial BW, kg	559.3	556.6	559.8	558.8	7.3	0.6640
Final BW, kg	599.0	602.5	605.7	604.9	7.2	0.1578
ADG, kg/d	1.28[c]	1.48[b]	1.48[b]	1.49[b]	0.05	0.0027
Carcass ADG, kg/d[e]	0.96[d]	1.20[c]	1.20[c]	1.45[b]	0.04	<0.0001
DMI, kg/d	9.7[b]	9.5[bc]	9.3[c]	9.0[d]	0.2	0.0003
G:F	0.132[d]	0.156[c]	0.159[c]	0.165[b]	0.04	<0.0001
Carcass G:F[e]	0.099[d]	0.126[c]	0.129[c]	0.160[b]	0.005	<0.0001

[a] Control – No Beta-Agonist; RAC 300 –Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 –RH at 400 mg/hd/d; Zilpaterol –Zilpaterol hydrochloride at 6.8 g/t.

[b-d] Least squares means within a row lacking a common superscript differ ($P < 0.05$).

[e] Initial HCW = 0.2491*Initial BW$^{1.1415}$ (Tatum et al., 2012).

Table 4.3. Least squares means for carcass characteristics of calf-fed Holstein steers implanted with Synovex®-C and Revalor®-XS and finished with or without supplementation in the diet with beta-agonists.

	Treatment[a]					
	Control	RAC300	RAC400	Zilpaterol	SEM	P_{TRT}
No. of Pens	8	8	8	8	-	-
HCW, kg	370.9[d]	376.8[c]	378.6[c]	385.5[b]	6.0	<0.0001
DP[f], %	61.9[d]	62.5[c]	62.5[c]	63.7[b]	0.17	<0.0001
LM area, cm^2	77.2[d]	80.2[c]	80.0[c]	85.5[b]	0.8	<0.0001
AFAT, cm	0.80[b]	0.76[c]	0.77[c]	0.73[d]	0.02	0.0065
KPH, %	2.93	2.83	2.79	2.62	0.08	0.0702
YG	2.35[b]	2.22[c]	2.23[c]	2.04[d]	0.04	<0.0001
Marbling Score	421	410	419	413	9	0.1746
HCW>431 kg, %	2.8[c]	4.1[c]	2.9[c]	7.0[b]	2.0	<0.0001
HCW>476 kg, %	0.00	0.14	0.00	0.42	-	-
Liver Abscess[g] (A), %	8.3	10.9	9.6	7.2	1.6	0.0690
Liver Abscess[g] (A+), %	7.8	9.2	6.8	6.6	2.0	0.4319
Total Liver Abscess, %	16.1[c]	20.1[b]	16.5[c]	13.8[c]	2.9	0.0133

[a] Control – No Beta-Agonist; RAC 300 –Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 –RH at 400 mg/hd/d; Zilpaterol –Zilpaterol hydrochloride at 6.8 g/t.

[b-e] Least squares means within a row lacking a common superscript differ ($P < 0.05$).

[f] DP = Dressing Percentage.

[g] A = 1-2 abscesses; A+ = multiple large abscesses.

Table 4.4. Probability [P] of various yield grades (YG) and quality grades (QG) as determined by VBG 2000 VIA system data from calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists.

	Treatment[a]					
	Control	RAC300	RAC400	Zilpaterol	SEM	P_{TRT}
Calculated YG						
YG 1	26.7[d]	37.6[c]	39.6[c]	58.2[b]	4.6	<0.0001
YG 2	70.8[b]	57.3[c]	60.4[c]	41.4[d]	4.2	<0.0001
YG 3	1.6	1.3	1.1	0.1	0.8	0.0739
YG 4[e]	-	-	-	-	-	-
Calculated QG						
Prime[f]	0.0	0.3	1.5	0.9	-	-
Upper 2/3 Choice	19.5[b]	13.5[c]	15.1[c]	15.3[c]	3.0	0.0175
Lower 1/3 Choice	43.7	43.2	44.3	41.1	3.2	0.6967
Select	34.9[c]	41.2[b]	37.4[cb]	41.0[b]	5.0	0.0536
No Roll	1.0	1.2	0.8	1.0	0.4	0.9488

[a] Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

[b-d] Least squares means within a row lacking a common superscript differ ($P < 0.05$).

[e] No carcasses calculated to be YG 4 or YG 5.

[f] No carcasses within the control treatment qualified as Prime, consequently probabilities were unable to be separated in a general linear mixed model.

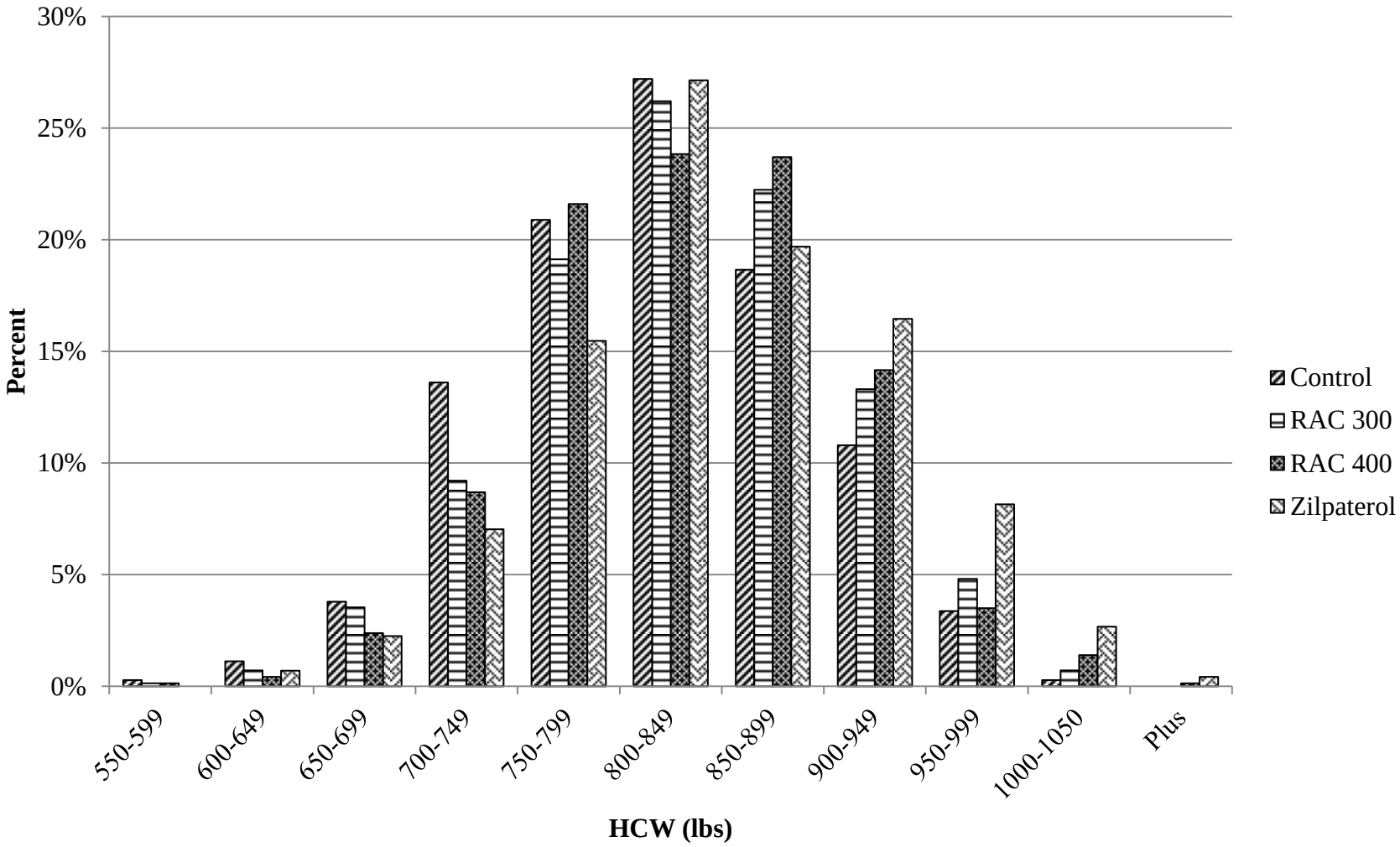

Figure 4.1. Hot carcass weight (HCW) distribution for carcasses from calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists. Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

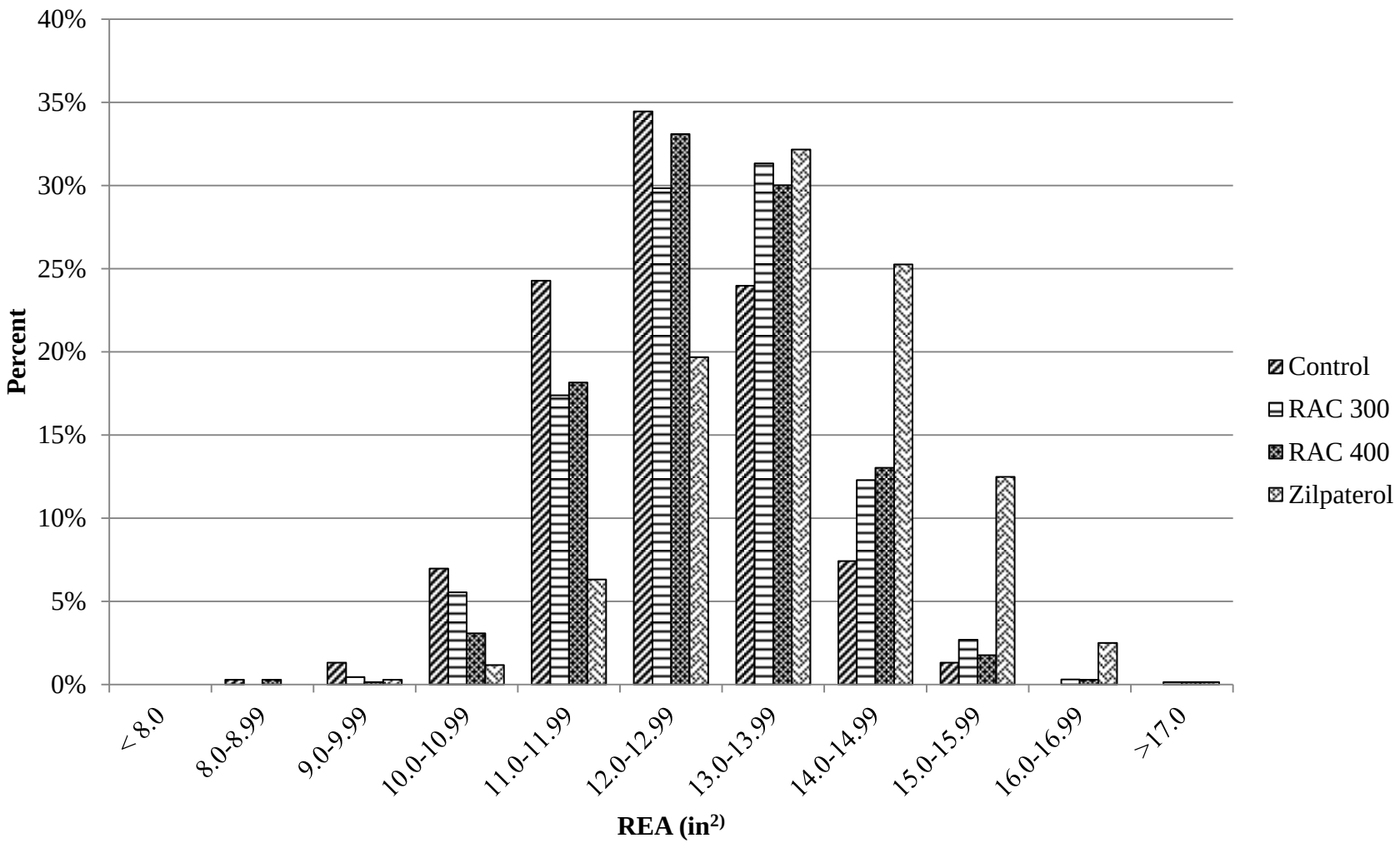

Figure 4.2. Ribeye area (REA) distribution for carcasses from calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists. Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

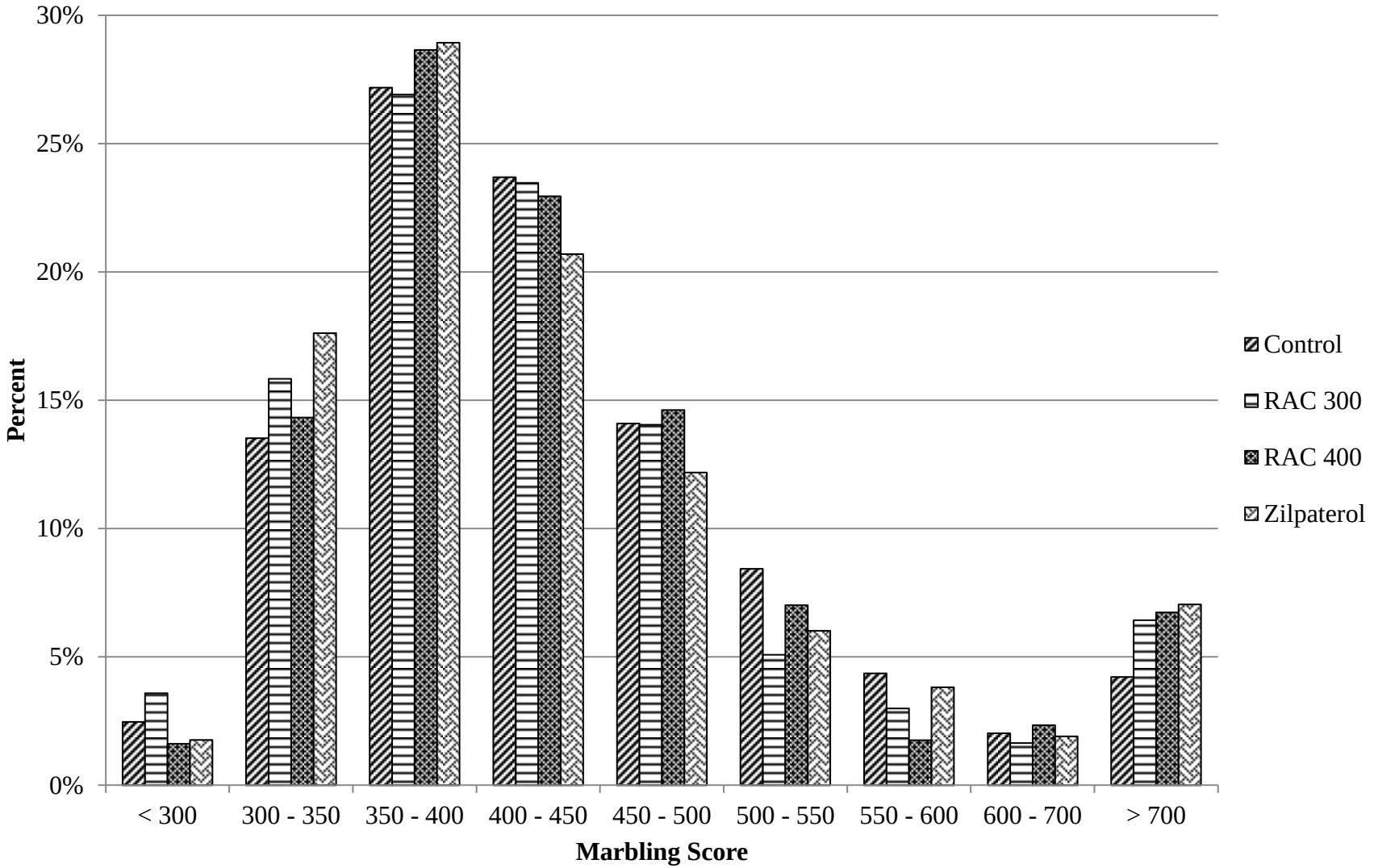

Figure 4.3. Marbling score distribution for carcasses from calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists. Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

Table 4.5. Percent of chilled side weight comprised of saleable yield, trim, fat and bone in carcasses from calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists.

	Treatment[a]					
	Control	RAC300	RAC400	Zilpaterol	SEM	P_{TRT}
Subprimal Yield[b]	0.00[e]	0.61[d]	0.86[d]	1.96[c]	0.18	<0.0001
Round	0.00[e]	0.22[e]	0.54[d]	1.0[c]	0.12	<0.0001
Loin	0.00[d]	-0.07[d]	0.10[cd]	0.23[c]	0.06	0.0141
Rib	0.00	-0.22	-0.24	-0.24	0.08	0.1037
Chuck	0.00[cd]	0.04[c]	-0.28[de]	-0.52[e]	0.11	0.0017
Forequarter	0.00[c]	-0.18[c]	-0.51[d]	-0.76[d]	0.11	0.0002
Hindquarter	0.00[e]	0.14[e]	0.64[d]	1.22[c]	0.12	<0.0001
Trim	0.00	0.04	0.01	-0.02	0.24	0.9620
Fat	0.00[c]	-0.56[c]	-0.63[c]	-1.32[d]	0.25	0.0054
Bone	0.00[c]	-0.17[c]	-0.30[cd]	-0.69[d]	0.19	0.0106

[a] Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

[b] Saleable yield from whole muscle cuts, division by primal represents percent of total saleable yield from each major primal.

[c-f] Least squares means within a row lacking a common superscript differ ($P < 0.05$).

Table 4.6. Subprimal yield of carcasses from calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists. Values expressed as a percent change from control of total chilled side weight.

	Treatment[a]					
	Control	RAC 300	RAC 400	Zilpaterol	SEM	P_{TRT}
Ribeye Roll	0.00[c]	0.02[c]	0.07[bc]	0.11[b]	0.03	0.0293
Back Ribs	0.00	-0.01	-0.01	-0.03	0.02	0.1668
Short Rib	0.00	0.00	0.00	-0.02	0.03	0.3457
Lifter Meat	0.00[c]	0.02[c]	0.02[c]	0.10[b]	0.02	0.0004
Hanging Tender	0.00	-0.05	-0.07	0.00	0.02	0.0839
Rib Fingers	0.00	-0.01	0.00	0.00	0.01	0.9530
Total Rib Fat	0.00	-0.11	-0.10	-0.12	0.08	0.1916
Total Rib Trim	0.00	-0.05	-0.05	-0.11	0.10	0.1300
Total Rib Bone	0.00	-0.04	-0.05	-0.09	0.03	0.0603
Navel	0.00[b]	-0.05[bc]	-0.09[cd]	-0.16[d]	0.04	0.0093
Outside Skirt	0.00	0.01	0.01	0.00	0.01	0.1093
Inside Skirt	0.00	0.01	0.02	0.03	0.01	0.0765
Strip Loin	0.00	-0.03	0.03	0.14	0.02	0.2345
Top Butt	0.00[d]	0.01[cd]	0.07[c]	0.16[b]	0.03	<0.0001
Tri-Tip	0.00[d]	0.04[c]	0.04[c]	0.07[b]	0.01	0.0002
Flap Meat	0.00	0.03	0.03	0.02	0.01	0.0864
Tenderloin	0.00[c]	0.02[c]	0.03[c]	0.08[b]	0.02	0.0080
Flank	0.00[d]	0.01[cd]	0.02[c]	0.04[b]	0.01	0.0003
Rose Meat	0.00	0.05	0.04	0.06	0.03	0.1361
Total Loin Fat	0.00[b]	-0.22[c]	-0.16[c]	-0.46[d]	0.09	0.0014
Total Loin Trim	0.00	0.05	0.05	0.08	0.08	0.4019
Total Loin Bone	0.00	-0.02	-0.05	-0.09	0.03	0.2178
Chuck Eye	0.00	0.03	-0.01	0.06	0.06	0.4985
Bone-In Short Rib	0.00	0.01	0.01	-0.02	0.02	0.4425
Chuck Flap	0.00	0.00	-0.02	0.00	0.01	0.1200
Pectoral	0.00[c]	0.01[c]	0.01[c]	0.04[b]	0.01	0.0055
Shank Meat	0.00	0.03	0.02	0.00	0.05	0.8617
Clod	0.00[c]	0.10[b]	0.07[bc]	0.12[b]	0.03	0.0343
Chuck Tender	0.00[c]	0.01[bc]	0.02[b]	0.03[b]	0.01	0.0090
Teres Major	0.00[c]	0.02[b]	0.01[c]	0.03[b]	0.003	<0.0001
Brisket	0.00	0.03	0.01	0.04	0.03	0.3432
Total Chuck Trim	0.00	0.05	0.00	0.00	0.09	0.9312
Total Chuck Fat	0.00[b]	-0.05[bc]	-0.14[cd]	-0.21[d]	0.05	0.0033
Total Chuck Bone	0.00[b]	-0.04[b]	-0.14[bc]	-0.28[c]	0.07	0.0385
Top Round	0.00[d]	0.09[c]	0.16[c]	0.37[b]	0.03	<0.0001
Bottom Round	0.00[d]	0.07[cd]	0.11[c]	0.24[b]	0.03	<0.0001
Eye of Round	0.00[d]	0.03[cd]	0.07[c]	0.14[b]	0.02	<0.0001
Knuckle	0.00[d]	0.05[cd]	0.11[bc]	0.18[b]	0.03	0.0002

Heel	0.00[d]	0.01[cd]	0.03[c]	0.07[b]	0.01	<0.0001
SDF[e]	0.00[c]	0.00[bc]	0.01[b]	0.01[b]	0.004	0.0194
Round Shank	0.00[c]	0.02[c]	0.01[c]	0.08[b]	0.03	0.0418
Total Round Trim	0.00[c]	0.12[b]	0.17[b]	0.17[b]	0.04	0.0076
Total Round Fat	0.00[b]	-0.11[cd]	-0.08[c]	-0.21[d]	0.04	0.0068
Total Round Bone	0.00[b]	-0.04[b]	-0.04[b]	-0.19[c]	0.04	0.0026
100% Lean Trim[f]	0.00[d]	0.31[cd]	0.52[bc]	0.73[b]	0.21	0.0085

[a] Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

[b-d] Least squares means within a row lacking a commons superscript differ ($P < 0.05$).

[e] SDF = superficial digital flexor.

[f] 100% Lean Trim = (% trim of chilled side weight)*(trim % lean). Trim percent lean calculated based on output from a MeatMaster™ (FOSS, Hilleroed, Denmark).

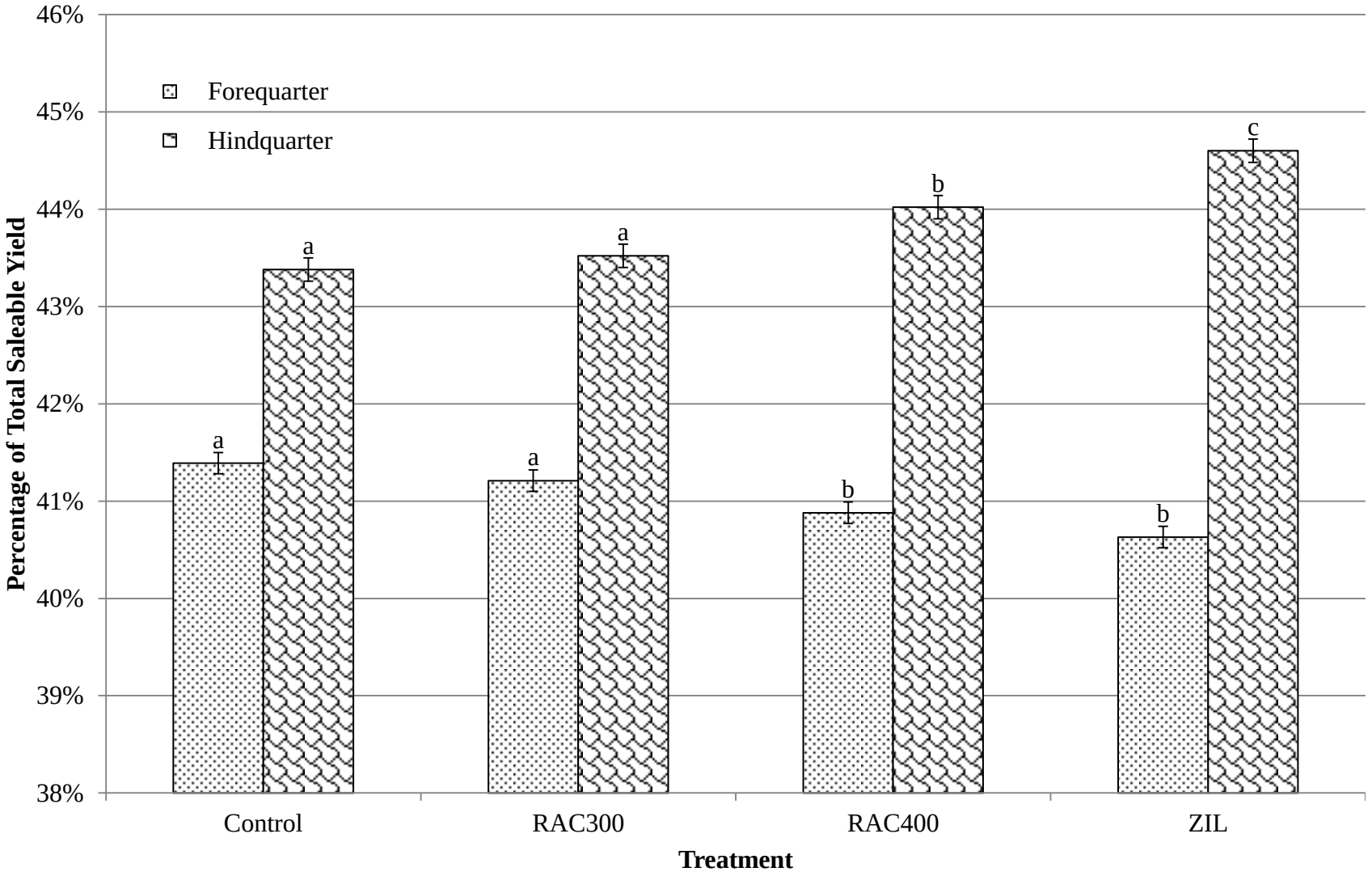

Figure 4.4. Percent of total saleable yield by quarter from calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists. Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

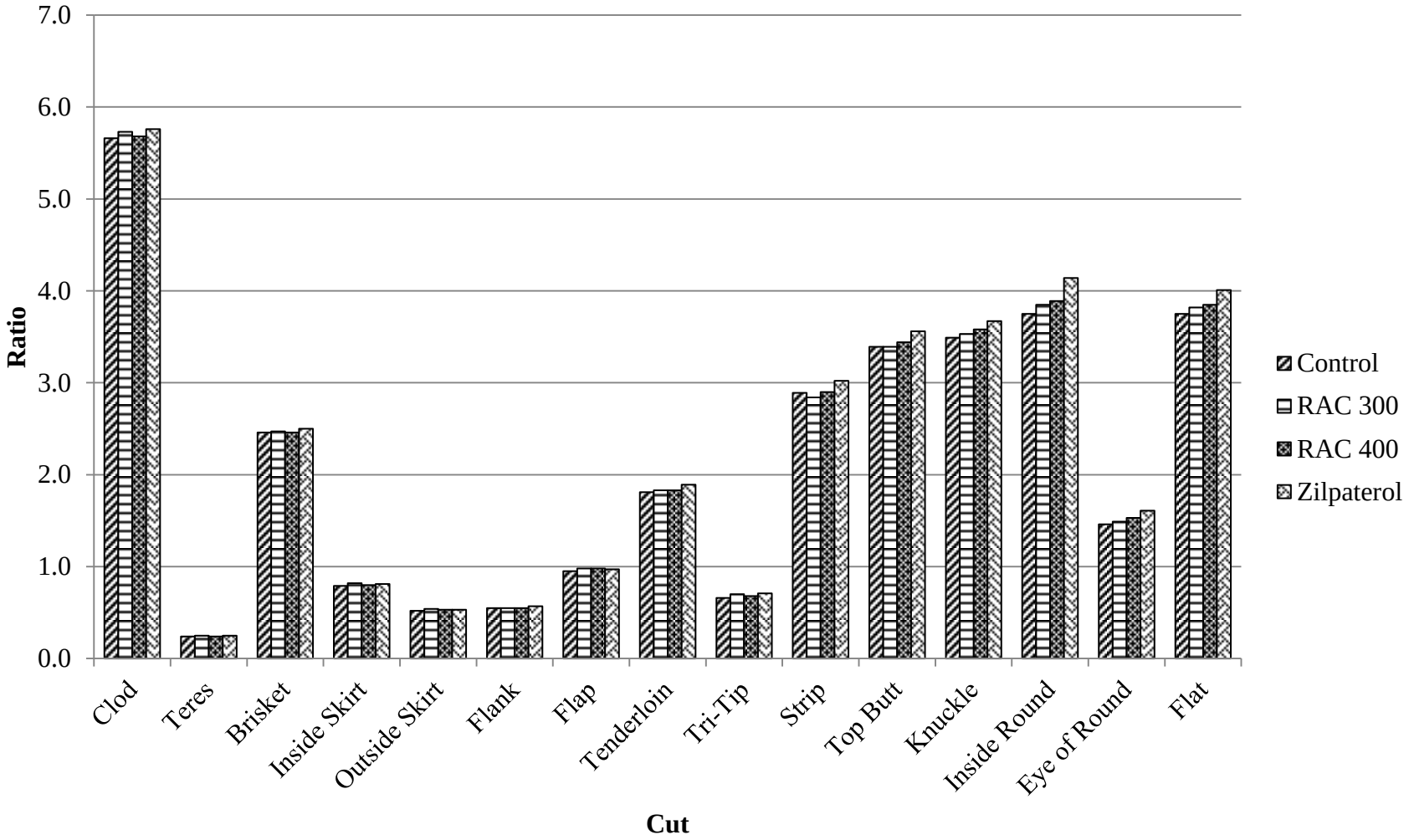

Figure 4.5. Ratio of various muscles to the *Supraspinatus* for carcasses from calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists. Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

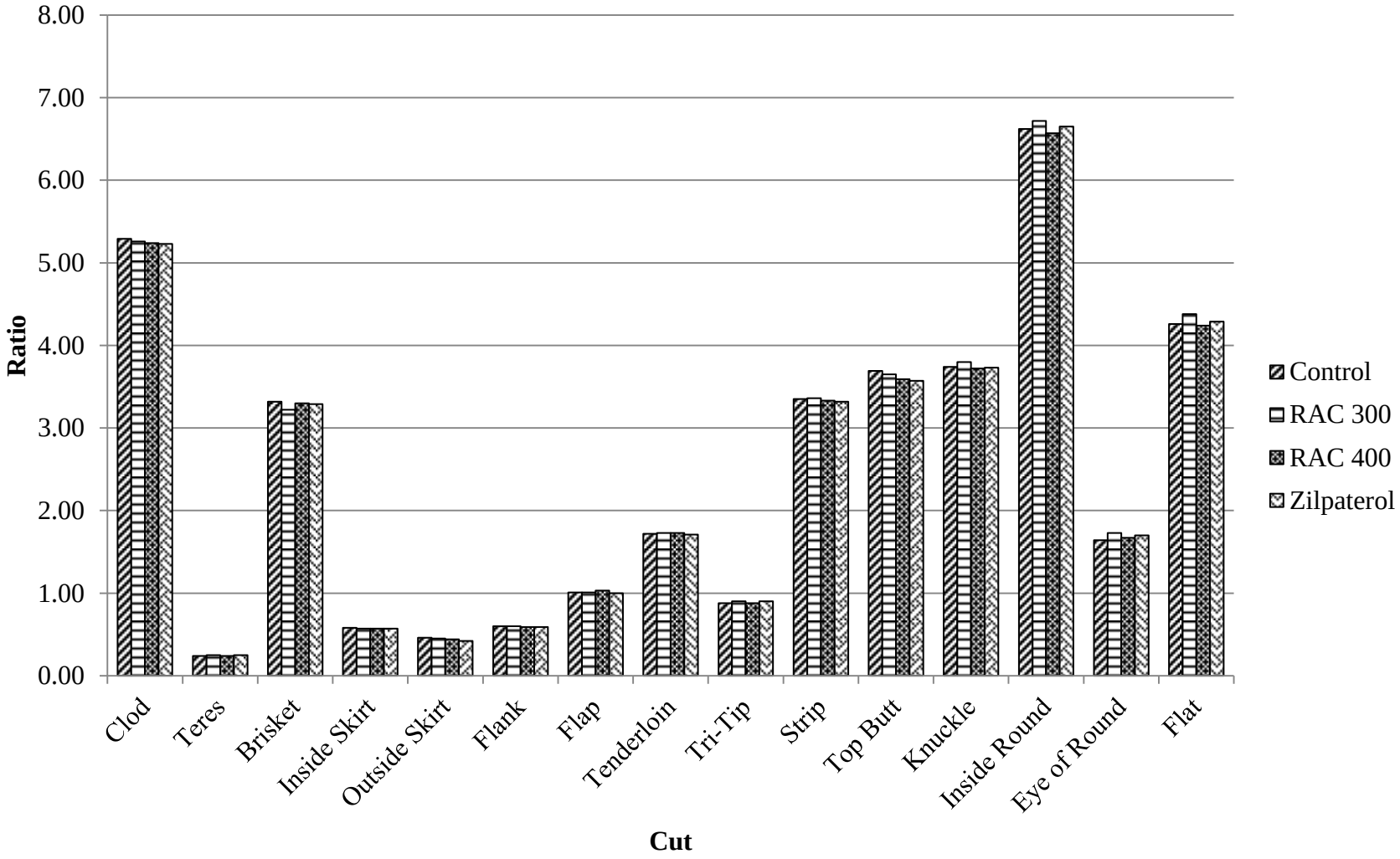

Figure 4.6. Ratio of various muscles to the *Supraspinatus* for carcasses from beef breeds of cattle managed with or without supplementation in the diet with beta-agonists. Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t. (Data from Arp, 2012).

Table 4.7. Slice shear force (SSF), probability [P] of failing to be certified as tender (SSF ≥ 20 kg) and cook loss of steaks from calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists.

	Treatment[a]					
	Control	RAC 300	RAC 400	Zilpaterol	SEM	P_{TRT}
14 d SSF, kg	16.3^{dx}	18.3^{cx}	18.9^{cx}	20.5^{bx}	0.4	<0.0001
[P] ≥ 20 kg[e]	0.16^{c}	0.31^{b}	0.33^{b}	0.42^{b}	0.05	0.0003
21 d SSF, kg	15.0^{dy}	16.4^{cy}	17.0^{bcy}	18.2^{by}	0.5	0.0007
[P] ≥ 20 kg[f]	0.12^{d}	0.15^{cd}	0.20^{bc}	0.28^{b}	0.04	0.0248
Cook Loss[g], %	16.5	16.6	16.5	17.0	0.5	0.2493

[a] Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

[b-d] Least squares means within a row lacking a common superscript differ ($P < 0.05$).

[e] Block effect removed from model due to non-significance ($P = 0.8266$), inclusion of block had no effect on estimates (±0.01), SEM, P_{TRT} or differences between treatments.

[f] Block effect removed from model due to non-significance ($P = 0.2982$), inclusion of block had no effect on estimates (±0.02), SEM, P_{TRT} or differences between treatments.

[x,y] Least squares means within a column lacking a common superscript differ ($P < 0.05$).

[z] Model includes peak internal temperature (degree of doneness) as a covariate.

Table 4.8. Probability [P] of top loin steaks failing to be certified as tender (slice shear force ≥ 20 kg) from various quality grades of carcasses of calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists.

	Treatment[a]					
	Control	RAC 300	RAC 400	Zilpaterol	SEM	P_{TRT}
Low Choice, 21 d[b]	0.07	0.19	0.19	0.21	0.06	0.2390
Low Choice, 14 d[b]	0.16	0.32	0.29	0.36	0.07	0.1255
Select, 21 d[c]	0.14[c]	0.17[c]	0.17[c]	0.39[b]	0.08	0.0511
Select, 14 d[d]	0.25	0.32	0.33	0.50	0.08	0.1199

[a] Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t

[b] Block effect removed from model due to non-significance (P = 0.85 – 0.60), inclusion of block had no effect on estimates (±0.01), SEM, P_{TRT} or differences between treatments.

[c] Block evaluated and found to be influential only due to inadequate sample size in one block as a result of samples removed from population following inadvertent freezing during shipment. When block was included in the model, P_{TRT} = 0.07 and SEM was increased 0.01. Relationships between controls and Zilpaterol treatment were constant, RAC 300 and control compared to zilpaterol had P = 0.07 when block was included versus P = 0.04 when block was excluded.

[d] Block effect removed from model due to non-significance (P = 0.3785), inclusion of block reduced estimate for RAC 300 by 0.03, RAC 400 by 0.04 and Zilpaterol by 0.02. Standard error, P_{TRT} and differences between treatments were unchanged as a result of including block.

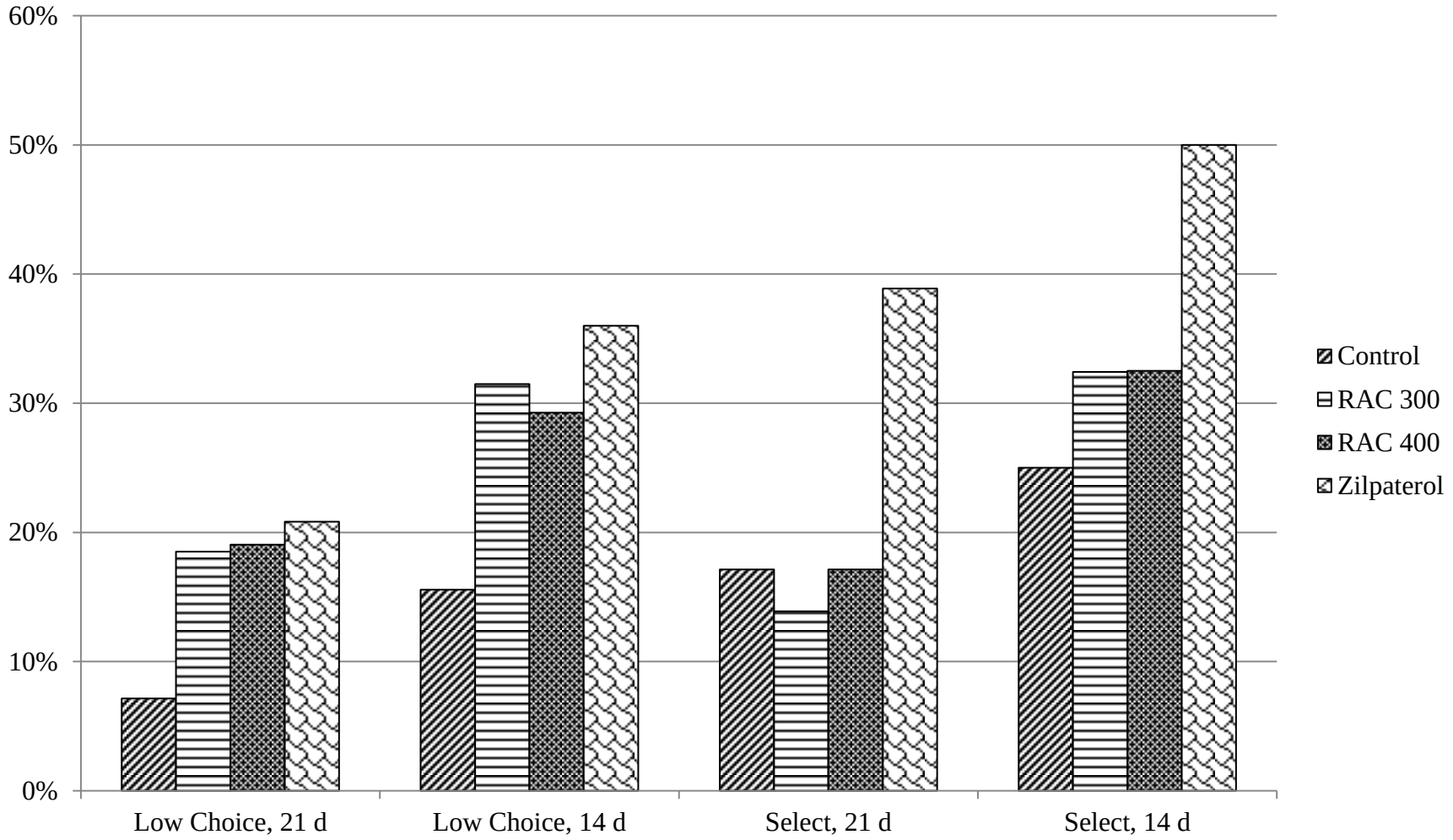

Figure 4.7. Frequency distribution of steaks with slice shear force > 20 kg from calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists. Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

Table 4.9. Slice shear force (SSF) of top loin steaks from calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists, segregated by quality grade, treatment and aging period.

14 d SSF, kg				21 d SSF, kg			
Treatment[a]	LSMean	SEM	N	Treatment[a]	LSMean	SEM	N
Control CH+	15.6[b]	1.1	23	RAC 300 CH+	14.0[b]	1.4	11
Control CH-	16.3[b]	0.8	45	Control CH+	14.4[b]	1.0	21
Control SE	16.7[bc]	0.9	36	Control CH-	15.0[b]	0.8	42
RAC 300 CH+	17.6[bcd]	1.5	13	Control SE	15.7[bc]	0.8	35
RAC 300 CH-	17.7[bcd]	0.7	54	RAC 400 CH-	15.8[bc]	0.8	38
RAC 400 CH+	18.0[bcd]	1.5	12	RAC 400 CH+	16.6[bcd]	1.4	10
RAC 400 CH-	18.1[bcd]	0.9	36	RAC 400 SE	16.7[bc]	0.9	32
Zilpaterol CH+	19.2[cd]	1.1	24	RAC 300 SE	16.7[bc]	0.8	36
Zilpaterol CH-	19.6[d]	0.8	50	RAC 300 CH-	16.7[bc]	0.7	54
RAC 400 SE	19.6[d]	0.9	36	Zilpaterol CH-	17.5[cd]	0.7	48
RAC 300 SE	19.6[d]	0.9	37	Zilpaterol CH+	17.9[cd]	1.0	21
Zilpaterol SE	22.3[e]	0.8	42	Zilpaterol SE	19.0[d]	0.8	36

[a] Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t. CH- = Low Choice; CH+ = Upper 2/3 Choice; SE = Select.

[b-e] Least squares means within a column lacking a common superscript differ ($P < 0.05$).

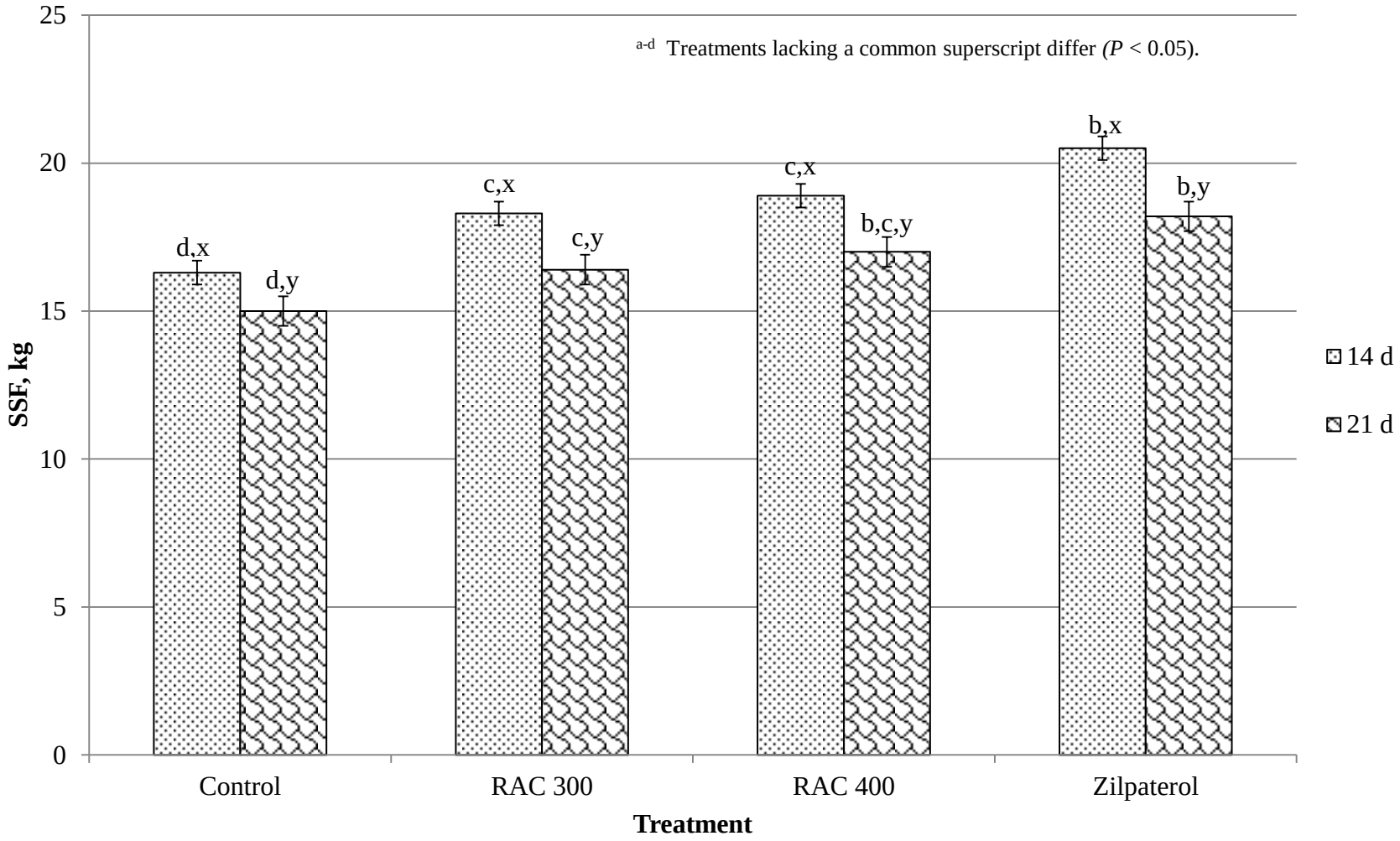

Figure 4.8. Slice shear force (SSF) of top loin steaks 14 and 21 d postmortem from calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists. Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

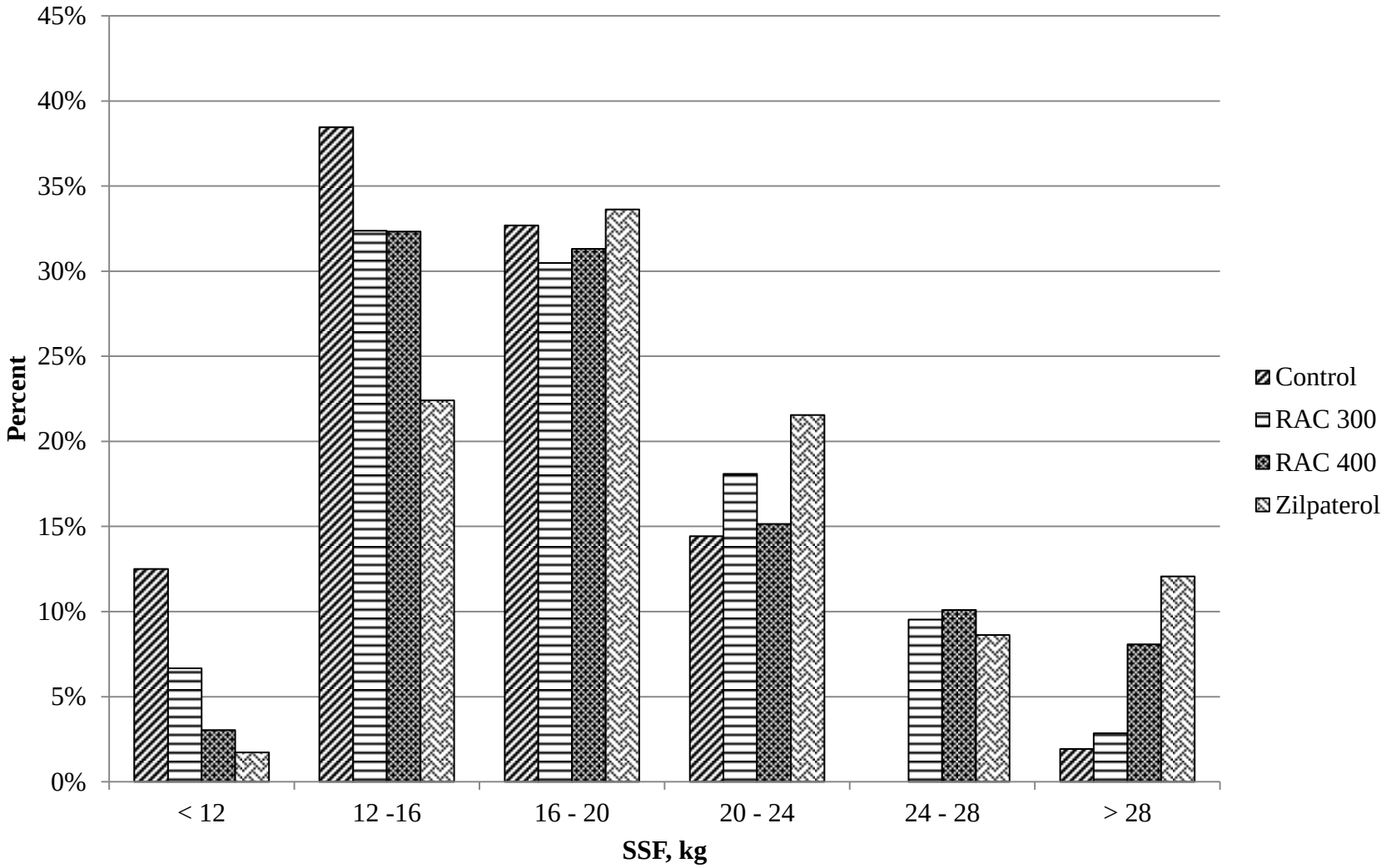

Figure 4.9. Slice shear force (SSF) for top loin steaks aged 14 d postmortem from calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists. Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

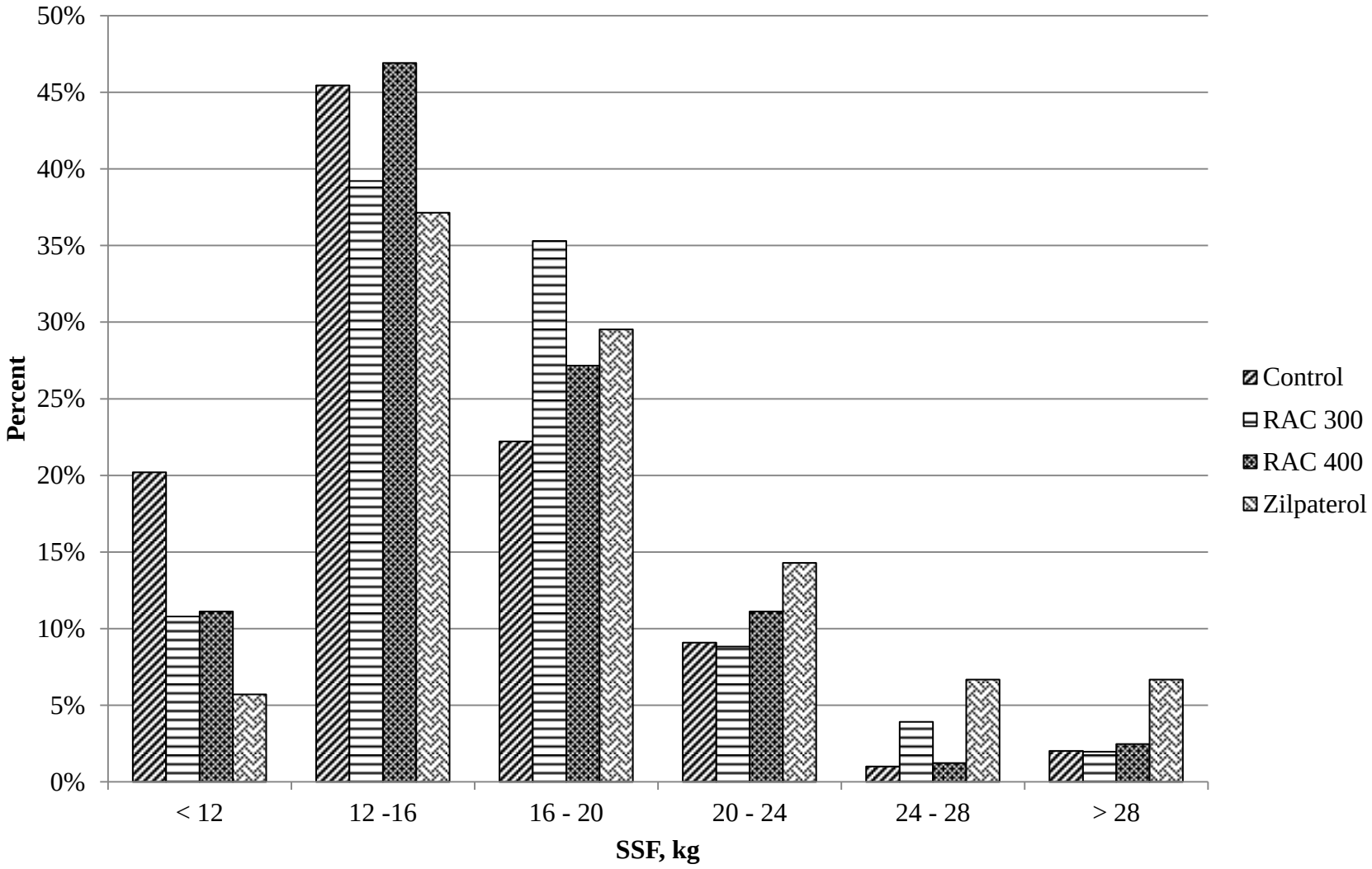

Figure 4.10. Slice shear force (SSF) for top loin steaks aged 14 d postmortem from calf-fed Holstein steers managed with or without beta-agonists. Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

Table 4.10. Trained sensory panel scores for top loin steaks from low Choice carcasses of calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists, 14 d postmortem.

	Treatment[a]				
Trait[b]	Control	RAC 300	Zilpaterol	SEM	P_{TRT}
Overall Tenderness	9.5[c]	8.8[cd]	8.3[d]	0.3	0.0109
Myofibrillar	9.5[c]	8.7[cd]	8.2[d]	0.3	0.0119
Connective Tissue	9.5[c]	9.0[cd]	8.4[d]	0.3	0.0400
Juiciness	8.0	7.7	7.8	0.2	0.2252
Beef Flavor[e]	8.4[cd]	8.6[c]	8.0[d]	0.2	0.0520
Buttery[e]	1.7	1.7	1.6	0.2	0.8835
Metallic	0.3	0.4	0.6	0.1	0.2813
Livery	0.08	0.06	0.01	0.03	0.1658

[a] Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

[b] Models include fixed effect of treatment and random effects of panel and harvest week, and covariates designated by superscript.

[c,d] Least squares means within a row lacking a common superscript differ ($P < 0.05$).

[e] Marbling score as assessed by a VIA system used as covariate. $P = 0.0217$ within model for flavor; $P = 0.0180$ within model for buttery.

Table 4.11. Trained sensory panel scores for top loin steaks from low Choice carcasses of calf-fed Holstein steers managed with or without supplementation in the diet with beta-agonists, 21 d postmortem.

	Treatment[a]				
Trait[b]	Control	RAC 300	Zilpaterol	SEM	P_{TRT}
Overall Tenderness	9.5[c]	9.7[c]	8.8[d]	0.2	0.0075
Myofibrillar	9.7[c]	9.7[c]	8.6[d]	0.3	0.0021
Connective Tissue	9.4	9.7	9.1	0.2	0.1933
Juiciness[e]	7.9[c]	8.1[c]	7.6[d]	0.2	0.0273
Beef Flavor[f]	8.9[c]	8.4[d]	8.1[d]	0.2	0.0044
Buttery[g]	2.0	1.7	1.5	0.2	0.1021
Metallic	0.3	0.5	0.5	0.1	0.4612
Livery	0.05	0.11	0.01	0.04	0.0920

[a] Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

[b] Models include fixed effect of treatment and random effects of panel and harvest week, and covariates designated by superscript.

[c-d] Least squares means within a row lacking a common superscript differ ($P < 0.05$).

[e] Peak temperature included as a covariate within the model ($P = < 0.0001$).

[f] Marbling score as assessed by a VIA system evaluated as a covariate and found to be non-significant ($P = 0.3205$).

[g] Marbling score as assessed by a VIA system used as covariate ($P = 0.0037$).

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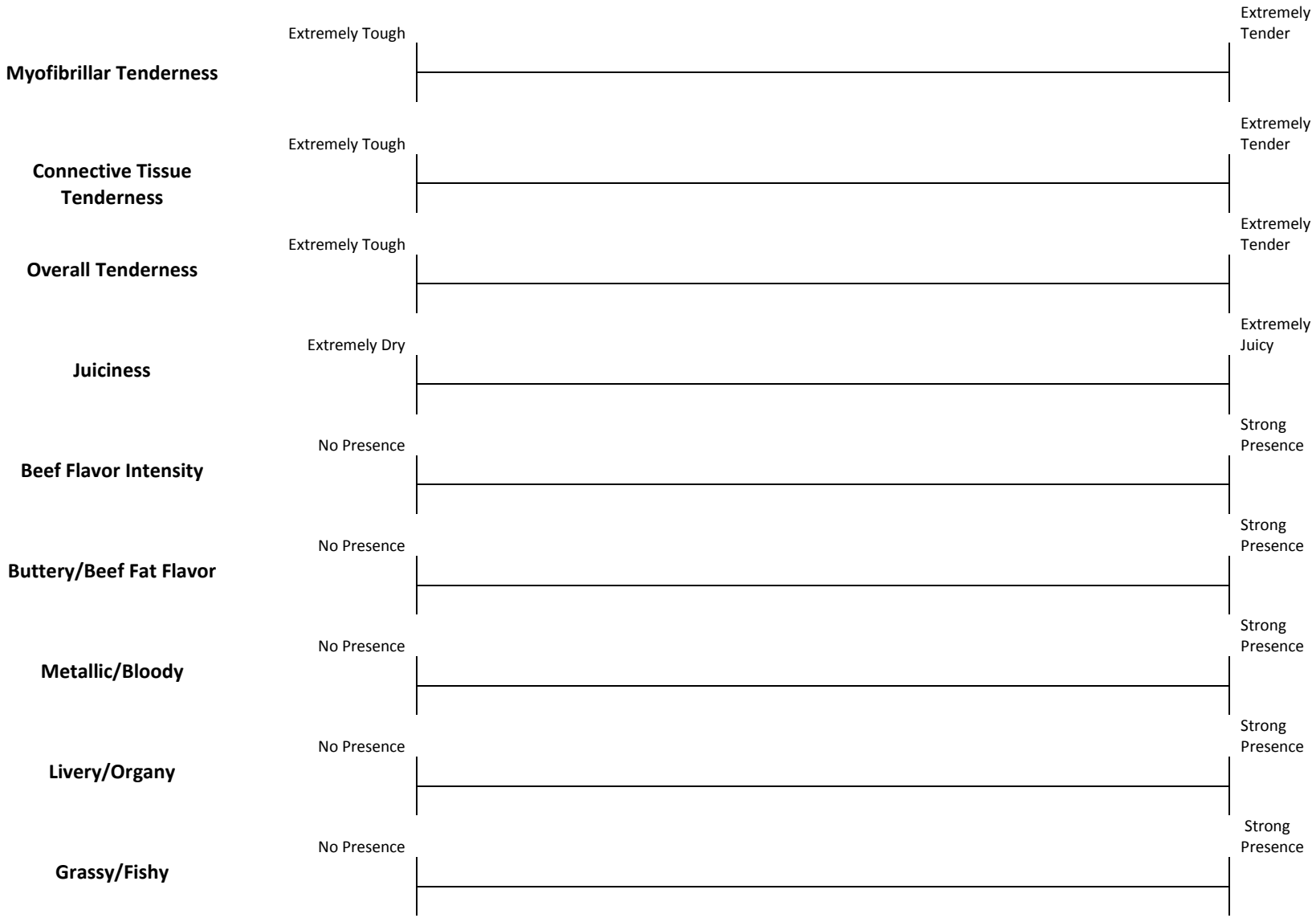
APPENDIX A

Table A.1. Description of items fabricated by primal.

Chuck	**Rib**	**Loin**	**Round**
Boneless Chuck Eye, 1 x 1	Boneless Ribeye, Lip-On, 2 x 2	Boneless Strip Loin, 0 x 1	Top Round, Cap Off
Chuck Flap	Backribs	Trim (50)	Bottom Round Flat S/T
Rope Meat (93)	Short Rib	Trim (85)	Bottom Round Eye S/T
Neck Meat (85)	Rib Finger Meat	Top Butt (1/4" Trim)	Superficial Digital Flexor
Bone/Cart/Backstrap	Hanging Tender	Peeled Tri-Tip	Bottom Round Heel
Bone-in Short Rib	Backstrap	Trim (85)	Shank Meat (85)
Pectoral Muscle	Lifter Meat	Peeled Tenderloin	Bell Knuckle
Shank Meat (85)	Trim	Flap Meat	Trim
Shoulder Clod (1/4" Trim)	Fat	Loin Tail	Fat
Teres Major	Bone	KPH	Bone
Paddle Bone		Trim	
Chuck Tender		Fat	
Trim		Bone	
Fat	**Plate**		
	9x22 Navel		
Brisket	Outside Skirt	**Flank**	
Boneless Brisket	Inside Skirt	Flank Steak	
Trim	Trim	Rose Meat	
Fat	Fat	Trim	
Bone	Bone	Fat	

APPENDIX B


Myofibrillar Tenderness
Extremely Tough
Extremely Tender
Connective Tissue Tenderness
Extremely Tough
Extremely Tender
Overall Tenderness
Extremely Tough
Extremely Tender
Juiciness
Extremely Dry
Extremely Juicy
Beef Flavor Intensity
No Presence
Strong Presence
Buttery/Beef Fat Flavor
No Presence
Strong Presence
Metallic/Bloody
No Presence
Strong Presence
Livery/Organy
No Presence
Strong Presence
Grassy/Fishy
No Presence
Strong Presence

APPENDIX C

Table C.1. Carcass and grade data for calf-fed Holstein steers managed without growth promotants or antibiotics (natural).

Mean Carcass Data		Grade Data Frequencies	
Trait	Mean	Quality/Yield Grade	Frequency, %
No. of Lots	4	Prime	7.1
HCW, kg	374.1	Upper 2/3 Choice	50.0
LM area, cm^2	71.0	Lower 1/3 Choice	35.1
AFAT, cm	1.03	Select	6.9
KPH, %	4.47	No Roll	0.0
Yield Grade (YG)	2.87	YG 1	1.7
Marbling Score	530	YG 2	65.7
HCW>431 kg, %	0.90	YG 3	32.5
HCW>476 kg, %	0.00	YG 4	1.5

Table C.2. Slice shear force (SSF), probability [P] of tough steaks and cook loss for steaks from calf-fed Holstein steers managed without growth promotants or antibiotics (natural).

	14 d SSF, kg		21 d SSF, kg	
Treatment	Mean	SD	Mean	SD
All Grades	14.0	3.5	13.5	3.3
SSF ≥ 20 kg, %	6.8	-	4.2	-
Natural CH^{+}	13.9	2.6	14.0	3.9
Natural CH^{-}	14.5	5.1	14.8	3.5
Cook Loss, %	16.2	3.3	16.5	3.2

Table C.3. Trained sensory panel ratings for steaks from calf-fed Holstein steers managed without growth promotants or antibiotics (natural).

	14 d Postmortem		21 d Postmortem	
	Mean	SD	Mean	SD
Overall Tenderness	10.6	0.8	10.6	0.9
Myofibrillar	10.5	0.7	10.4	0.8
Connective Tissue	10.5	0.7	10.5	0.9
Juiciness	8.1	0.7	8.3	0.7
Beef Flavor	8.9	0.6	8.8	0.7
Buttery	2.3	0.8	2.4	0.8
Metallic	0.4	0.3	0.2	0.2
Livery	0.1	0.2	0.1	0.2

APPENDIX D

Table D.1. Model parameter estimates for probability of slice shear force values > 20.0 kg in steaks from calf-fed Holstein steers managed with and without beta-agonists.

Model	Parameter[a]	Estimate	SE	95% Confidence Limits		$P\chi^2$
14d SSF	Intercept	-0.31	0.2	-0.68	0.06	0.0960
	Zilpaterol	0.00	0.0	0.00	0.00	-
	RAC 400	-0.38	0.3	-0.94	0.18	0.1809
	RAC 300	-0.51	0.3	-1.07	0.04	0.0708
	Control	-1.32	0.3	-1.96	-0.68	<0.0001
21d SSF	Intercept	-0.96	0.2	-1.39	-0.54	<0.0001
	Zilpaterol	0.00	0.0	0.00	0.00	-
	RAC 400	-0.41	0.3	-1.07	0.25	0.2243
	RAC 300	-0.79	0.4	-1.49	-0.10	0.0251
	Control	-1.02	0.4	-1.76	-0.27	0.0070
21d Low Choice	Intercept	-1.34	0.4	-2.03	-0.64	0.0002
	Zilpaterol	0.00	0.0	0.00	0.00	-
	RAC 400	-0.11	0.5	-1.15	0.93	0.8327
	RAC 300	-0.15	0.5	-1.12	0.83	0.7689
	Control	-1.23	0.7	-2.60	0.14	0.0775
14d Low Choice	Intercept	-0.57	0.3	-1.15	0.00	0.0508
	Zilpaterol	0.00	0.0	0.00	0.00	-
	RAC 400	-0.31	0.5	-1.19	0.58	0.4973
	RAC 300	-0.20	0.4	-1.02	0.61	0.6263
	Control	-1.12	0.5	-2.11	-0.12	0.0274
21d Select	Intercept	-0.45	0.3	-1.12	0.22	0.1862
	Zilpaterol	0.00	0.0	0.00	0.00	-
	RAC 400	-1.12	0.6	-2.23	-0.02	0.0463
	RAC 300	-1.37	0.6	-2.52	-0.21	0.0202
	Control	-1.12	0.6	-2.23	-0.02	0.0463
14d Select	Intercept	0.00	0.3	-0.60	0.60	1.0000
	Zilpaterol	0.00	0.0	0.00	0.00	-
	RAC 400	-0.73	0.5	-1.65	0.17	0.1100
	RAC 300	-0.73	0.5	-1.63	0.18	0.1164
	Control	-1.10	0.5	-2.07	-0.13	0.0260

[a] Control – Implanted with Revalor®-XS; RAC 300 – Revalor®-XS + Ractopamine hydrochloride (RH) at 300 mg/hd/d; RAC 400 – Revalor®-XS + RH at 400 mg/hd/d; Zilpaterol – Revalor®-XS + Zilpaterol hydrochloride at 6.8 g/t.

Test of hypotheses for treatments influencing probability of a steak exceeding 20 kg of SSF at 14 or 21 d postmortem required detailed statistical analysis to determine appropriate models and mean separation techniques. Diagnostics included determination of whether over dispersion was present in the count data for 14 and 21 d SSF > 20 kg. The statistical methodology (PROC GENMOD) used in this instance to assess count data assumed the model was correct and that variance was accurately estimated by the model. That is, the observed responses in data were comparable to the differences predicted by the model. If the model was appropriate, residual deviance (goodness-of-fit) would be distributed as χ^2. In a χ^2 distribution, an expected value should be approximately equal to degrees-of-freedom. Since deviance or goodness-of-fit was estimated by χ^2, if over dispersion was present or variance exceeded what is expected by the model, the Pearson χ^2 value divided by degrees of freedom would exceed one. In the present study, data separated by age and quality grade had values for Pearson χ^2 divided by degrees-of-freedom that ranged from 0.7 to 1.2 which indicated relatively appropriate models. Models utilized to assess data did not include the effect of block as the effect was non-significant ($P > 0.05$). Exclusion of the term could be viewed as inappropriate based on experimental design; however, inclusion of the term did not change the results of analysis for probability of steaks to exceed 20 kg SSF at 14 or 21 d postmortem. Including block in the model less-accurately reflected variance present in the data as determined by Pearson χ^2 divided by degrees of freedom. This would stand to reason since the block term was non-significant and including it in the model would partition a proportionally smaller amount of sums-of-squares away from the error term relative to the reduction in degrees-of-freedom.

APPENDIX E

Table E.1. Least squares means and standard error for sensory attributes of steaks from cattle of various type and quality grade, 14 d postmortem.

	Dairy – High Choice	Dairy – Low Choice	Certified Angus Beef	Low Choice Beef Breeds	SEM	*P*
Overall Tenderness	10.0[a]	9.2[b]	8.9[bc]	8.6[c]	0.2	<0.0001
Myofibrillar	9.8[a]	8.8[a]	8.5[ab]	8.1[b]	0.2	<0.0001
Connective Tissue	10.6[a]	10.3[a]	10.1[ab]	9.7[b]	0.2	0.0043
Juiciness	8.6[a]	8.0[b]	8.1[b]	7.9[b]	0.1	<0.0001
Beef Flavor	8.8[a]	8.7[a]	8.7[ab]	8.4[b]	0.2	0.0534
Buttery	3.0[a]	2.3[b]	2.3[b]	1.8[c]	0.1	<0.0001
SSF, kg	13.5[b]	15.1[b]	15.6[ab]	17.6[a]	0.9	0.0094
Cook Loss, %	17.3	17.7	18.1	18.1	0.6	0.4855

[a-c] LSMeans within a row lacking a common superscript differ ($P < 0.05$).

Table E.2. Least squares means for sensory attributes and slice shear force (SSF) of steaks from cattle of various type and quality grades, 21 d postmortem.

	Dairy – High Choice	Dairy – Low Choice	Certified Angus Beef	Low Choice Beef Breeds	SEM	*P*
Overall Tenderness	10.1[a]	9.7[ab]	9.5[b]	9.0[c]	0.2	0.0005
Myofibrillar	9.9[a]	9.3[b]	9.3[b]	8.7[c]	0.2	0.0002
Connective Tissue	10.8[a]	10.8[a]	10.5[ab]	10.1[b]	0.2	0.0063
Juiciness	8.6[a]	8.1[b]	8.1[b]	8.0[b]	0.1	0.0021
Beef Flavor	9.1[a]	8.6[bc]	8.8[ab]	8.5[c]	0.1	0.0018
Buttery	3.2[a]	2.3[b]	2.5[b]	1.8[c]	0.1	<0.0001
SSF, kg	11.4[c]	13.4[b]	14.1[b]	15.6[a]	0.7	<0.0001
Cook Loss, %	17.1	17.2	18.1	18.1	0.4	0.3148

[a-c] LSMeans within a row lacking a common superscript differ ($P < 0.05$).

Table E.3. Tenderness by type, quality grade and aging treatment as assessed by overall taste panel ratings and slice shear force (SSF). Panel rating vs. SSF correlation = -0.49 ($P < 0.05$).

	Overall			SSF (kg)		
	21d	14d	SEM	21d	14d	SEM
Dairy – CH+	10.1^{ax}	10.0^{ax}	0.2	11.4^{x}	13.5^{y}	-
Dairy – CH-	9.7^{abx}	9.2^{by}	0.2	13.4^{ax}	15.1^{ay}	0.7
Certified Angus	9.5^{bx}	8.9^{bcy}	0.2	14.1^{abx}	15.6^{ax}	0.7
CH- Beef Breeds	9.0^{cx}	8.6^{cx}	0.2	15.6^{bx}	17.6^{by}	0.8

^{a,b,c} LSMeans within a column lacking a common superscript differ ($P < 0.05$). No differences in SSF between Dairy – CH+ and other treatments were calculated due the fact Dairy – CH+ were sheared on a separate day from all other treatments.

^{x,y} LSMeans within a row lacking a common superscript differ ($P < 0.05$).

Table E.4. LSMeans for sensory attributes, slice shear force (SSF) and cook loss of steaks from calf-fed Holstein and beef breed type carcasses fed to trained sensory panelists, 14d postmortem.

	Holstein	Beef Type	SEM	*P*
Overall Tenderness	9.6	8.7	0.1	<0.0001
Myofibrillar	9.3	8.3	0.2	<0.0001
Connective Tissue	10.5	9.9	0.1	0.0023
Juiciness	8.3	8.0	0.1	0.0052
Beef Flavor	8.8	8.6	0.1	0.0330
Buttery	2.6	2.1	0.1	<0.0001
SSF	14.5	16.4	0.6	0.0146
[P] SSF > 20 kg	0.14	0.18	0.1	0.5190
Cook Loss, %	17.5	18.1	0.4	0.1621

Table E.5. LSMeans for sensory attributes, slice shear force (SSF) and cook loss of steaks from calf-fed Holstein and beef breed type carcasses fed to trained sensory panelists, 21d postmortem.

	Holstein	Beef Type	SEM	*P*
Overall Tenderness	9.9	9.3	0.1	0.0009
Myofibrillar	9.6	9.0	0.1	0.0011
Connective Tissue	10.8	10.3	0.1	0.0021
Juiciness	8.4	8.1	0.1	0.0151
Beef Flavor	8.9	8.7	0.1	0.0691
Buttery	2.7	2.2	0.1	<0.0001
SSF	12.7	14.8	0.4	0.0004
[P] SSF > 20 kg	0.05	0.07	0.03	0.6662
Cook Loss, %	17.1	18.1	0.3	0.0219