EFFECTS OF SUPPLEMENTING FEEDLOT STEERS AND HEIFERS WITH ZILPATEROL HYDROCHLORIDE ON WARNER-BRATZLER SHEAR FORCE OF STEER LONGISSIMUS LUMBORUM AND HEIFER LONGISSIMUS LUMBORUM, TRICEPS BRACHII AND GLUTEUS MEDIUS MUSCLES AGED FOR 7, 14 AND 21 DAYS

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Abstract

The longissimus lumborum (LL) muscle from 117 steers and the LL, gluteus medius (GM), and triceps brachii (TB) from 132 heifers were obtained to evaluate the effects of feeding zilpaterol hydrochloride (Zilmax®; ZH) (7.56g/907kg on a 100% DM basis) on tenderness. Both genders were blocked by initial weight into 6 blocks of 4 pens. Pens were assigned to treatments of either 0, 20, 30 or 40 d on ZH, with a 3 d withdrawal. One steak was removed from each muscle for proximate analysis and three 2.54 cm thick steaks were vacuum aged for either 7, 14 or 21 d. Steaks were cooked to 70 °C and six 1.27 cm diameter cores were removed for Warner-Bratzler shear force (WBSF) determinations. All muscles from steers and heifers fed 30 and 40 d with ZH, had higher (P < 0.05) WBSF compared with controls. The WBSF of steer LL and heifer TB from the 20 d treatment was higher (P < 0.05) than controls. There were no treatment by aging interactions (P > 0.05) for WBSF of steaks from steer LL, heifer LL or heifer TB, but there was a treatment by aging interaction (P < 0.05) for WBSF of steaks from heifer GM. There were no differences (P > 0.05) in percent intramuscular fat for any muscle due to treatment. When using percent intramuscular fat as a covariate, differences in WBSF of steer LL and heifer TB were not altered, but there were slight differences in heifer LL and GM WBSF due to treatment when compared with not using percent fat as a covariate. Percentages of steaks with WBSF ≥ 5 kg increased as days on ZH increased and decreased as days of aging increased. Warner-Bratzler shear force values among the three aging times for steer LL control, 20 and 40 d treatments; all heifer LL treatments, and heifer TB 20 d were all positively correlated (P < 0.01) with each other. Feeding ZH for 20 d generally increased WBSF values, but means were still acceptable. Feeding ZH for 40 d was very detrimental to tenderness.

Table of Contents

List of Figuresiv
List of Tablesv
Acknowlegementsvii
Dedicationviii
General Introduction1
CHAPTER 1-Literature Review2
Beta agonists2
Zilpaterol5
Tenderness6
References11
CHAPTER 2-Effects of supplementing feedlot steers and heifers with zilpaterol hydrochloride on
warner bratzler shear force of steer longissimus lumborum and heifer longissimus lumborum,
triceps brachii and gluteus medius muscles aged for 7, 14 and 21 d16
Introduction16
Materials and Methods17
Results and Discussion22
Summary39
References42
Appendix A

List of Figures

Figure 1. Effects of ZH treatment on WBSF of steer LL, heifer LL and heifer TB muscle
24
Figure 2. Interaction between aging and ZH treatment on WBSF of heifer GM muscle.26
Figure 3. Effect of aging on WBSF of steer LL muscle27
Figure 4. The effect of different aging times on WBSF of heifer LL and TB
muscles28
Figure 5. Effects of ZH treatment on WBSF of steer LL, heifer LL and heifer TB muscle
using % IMF as a covariate31
Figure 6. Effects of feeding ZH and aging on heifer GM muscle using % IMF as a
covariate32

List of Tables

Table 1. (Correlations among % IMF and WBSF of C steer LL muscle aged 7, 14 and 21	
(d3	3
Table 2. 0	Correlations among % IMF and WBSF of heifer LL muscle aged 7, 14 and 21	
(d3	4
Table 3. 0	Correlations among % IMF and WBSF of heifer TB muscle aged 7, 14 and 21	
(d3	5
Table 4. 0	Correlations among % IMF and WBSF of heifer GM muscle aged 7, 14 and 21	
(d3	6
Table 5. 0	Correlations among WBSF of C heifer LL, TB and GM muscle aged 7, 14 and 21	
(d3	7
Table 6. 0	Correlations among WBSF of 20 d heifer LL, TB and GM muscle aged 7, 14 and 21	
(d3	7
Table 7. 0	Correlations among WBSF of 30 d heifer LL, TB and GM muscle aged 7, 14 and 21	
(d3	8
Table 8. 0	Correlations among WBSF of 40 d heifer LL, TB and GM muscle aged 7, 14 and 21	
(d3	9

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Dedication

I would like to dedicate this thesis to my grandfather Richard Claus Sr. Thank you for your support and always believing in me.

General Introduction

In order to improve efficiency in the beef cattle industry, growth promoting agents are being used to enhance lean gain efficiency. There are several management strategies used to maximize the efficiency of production, meat yield, and quality of meat. One of these management strategies that improves efficiency of growth and increases amount of carcass lean is the use of a class of feed additives called beta-adrenergic agonists (β-AA) (Beerman, 2004). Beta-adrenergic agonists are in a category called metabolic modifiers. The definition of a metabolic modifier is a compound that is either fed, injected or implanted in animals to improve rate of gain, improve feed efficiency, increase dressing percent, increase carcass meat yield percentage, improve visual meat quality, extend shelf-life, alter meat nutritional profile, or improve meat palatability (Dikeman, 2007). These compounds have been studied extensively in the past few decades, but only ractopamine-HCL and zilpaterol-HCL are approved for use in meat animals. These compounds repartition nutrients away from fat deposition, and towards muscle growth. According to Anderson (2000), any drug that affects efficiency of production works through one of three mechanisms: increased energy consumption above maintenance, altered composition through reduced energy content of the empty body, or through improved efficiency of the processes that result in growth.

Chapter 1- Literature Review

Beta Agonists

Mode of Action

Beta-adrenergic agonists are a group of phenethanolamines that act as potent growth promoters causing a dramatic increase in skeletal muscle while decreasing body fat content (Beerman et al., 1986; Mersmann, 1998). These growth promoters are repartitioning agents that direct nutrients away from fat synthesis towards muscle protein synthesis. Beta-adrenergic agonists are orally active and are generally fed at the end of the finishing stage (Mersmann, 1998). Beta-agonist receptors are present in all organs that are associated with growth, such as skeletal muscle and adipose tissue (Yang and McElligott, 1989). There are three subtypes of β-AA receptors: β₁, β₂ and β₃. The primary receptor subtype in muscle has been identified as being β₂, although bovine skeletal muscle was also found to have a small population of β₁-receptors (Sillence and Matthews, 1994). A physiological response occurs when a β-AA, such as the catecholamines, norepinephrine and epinephrine, bind to receptors and cause a cascade of events that amplifies the original signal. Once a β-AA is bound to its receptor, a G stimulatory protein is stimulated, which activates adenylyl cyclase. Activated adenylate cyclase is an enzyme that catalyses the conversion of ATP to cAMP, which binds to protein kinase A. Activated protein kinase A will phosphorylate several intracellular proteins leading to either activation or inactivation. For example, hormone sensitive lipase is activated in fat cells, which increases lipolysis and inhibits acetyl-CoA carboxylase, which in turn, inhibits long-chain fatty acid biosynthesis (Mersmann, 1998). After the receptor is activated, the catecholamine needs to be removed so the receptor

doesn't remain activated. Catechol-o-methyl transferase inactivates catecholamines and they can be reabsorbed (Mersmann, 1998).

Effects on growth

The growth effects that β-AA have on muscle appear to be the result of true muscle hypertrophy. Beerman et al. (1986) determined that muscle DNA concentration decreased in cimaterol treated lambs, while the overall DNA content did not change. This indicated that the growth of muscle was most likely due to hypertrophy and not the division of satellite cells. Beta- adrenergic agonist affects on muscle have been shown to be transient; if they are fed long term, their effectiveness decreases. McElligott et al. (1989) determined, after feeding clenbuterol to rats, that an effect started to take place after 2 d and the effect reaches a maximum after 8 d. After 14 d of treatment, the effect started to attenuate until ADG was the same as controls. It was suggested that this may be due to the down-regulation of β-AA receptors. Yang and McElligott (1989), Kim and Sainz (1992), and Moloney et al. (1991) demonstrated that an increase in muscle protein synthesis and a decrease in muscle protein degradation are what lead to the overall increase in muscle mass. Mersmann (1998) also determined that there may be other mechanisms of action of β-AA in which they increase blood flow to skeletal muscle and increase the amounts of substrates and energy for muscle hypertrophy. The increased blood flow to adipose tissue may increase lipid degradation by carrying nonesterified fatty acids away from the tissue. Another possible mechanism by which β-adrenergic agonists can increase muscle growth is by activating non-muscle β-receptors, which leads to an increase in circulating concentrations of hormones. These hormones can then act on the muscle or create an environment that is favorable for protein accretion (Yang and McElligott, 1989). Beta-adrenergic agonists have been shown to elevate plasma catecholamine levels in pigs which can then mediate growth in various tissues

(Mersmann, 1998). It may be a combination of these mechanisms and not just one that causes the effects of increased muscle mass.

There are several β-agonists that have been shown to have potential effects on muscle growth. Some of these are ractopamine-HCL (Optaflexx™), cimaterol, clenbuterol and zilpaterol-HCL (Zilmax®; ZH). Ractopamine and ZH are the only two of these compounds that are approved for use in meat animals (Beerman, 2004). Research has shown that cattle fed cimaterol or clenbuterol had muscles in the hind legs that were 25-30% larger and carcass fat was decreased by 15-25% (Beerman, 2004). Schiavetta et al. (1990) showed that steers fed clenbuterol for 50 d had increased (P < 0.05) cooked meat shear force by 19% over controls and decreased (P < 0.05) marbling scores. According to Avendano-Reyes et al. (2006), clenbuterol has caused acute toxicity in humans from consumption of viscera from clenbuterol fed animals. This led to its removal from the market. Both clenbuterol and cimaterol have long half-lives in animals, which contribute to the problem of significant residues left in the tissue that can be consumed by humans (Beerman, 2004). Zilpaterol and ractopamine are metabolized much more quickly and removed from the animal tissues. As a result, ZH and ractopamine are the two β-agonists that have been approved for use in meat animals (Avendano-Reyes et al., 2006). Ractopamine increased (p < 0.05) carcass yield in treated animals versus controls, but ribeye area was not increased (p > 0.05). Beerman (2004) indicated that cattle fed ractopamine contained 10 kg more lean muscle than controls, when equal amounts of feed were given. Platter et al. (2008) concluded that feeding ractopamine does not have a significant impact on marbling scores. They also concluded that it might slightly increase WBSF but the change would likely have a minimal impact on consumer acceptability. According to Avendano-Reyes et al. (2006), the feeding of ractopamine did lead to an increase in toughness of the longissimus muscle; however, shear values were similar between steers fed ZH and those fed

ractopamine. The authors indicated that ractopamine fed steers tended to have greater fat thickness than ZH fed steers and consumed less feed than control steers.

Zilpaterol

Zilpaterol is a synthetic β-agonist that improves growth performance, dressing percent and carcass muscling (Dikeman, 2003). Plascencia et al. (1999) determined that feeding ZH to steers increased dressing percentage (2.2 percentage points) and ribeye area (2.7%). Along with this beneficial effect on growth, Avendano-Reyes et al. (2006) and Beerman (2004) indicated that there was an increase (p < 0.01) in Warner-Bratzler shear force of longissimus muscles of steers fed ZH for 45 d, along with lower sensory tenderness and juiciness. Steers fed for 15 or 30 d had no negative effects on meat quality, so it was suggested that the length of time for feeding ZH to steers should be restricted to less than 45 d. Strydom et al. (2007) determined that loins from bulls fed ZH for 30 d had higher shear force (p < 0.05) than controls not fed ZH. Strydom and Nel (1999) also reported tougher longissimus steaks after feeding ZH for 30 and 50 d for carcasses that were not electrically stimulated. After electrical stimulation, and 10 d of aging, the differences were negated. Strydom and Nel (1999) also indicated that the effects of ZH on meat quality are muscle specific. Dikeman (2000) stated that ZH appears to have potential to improve efficiency of growth and carcass composition and will only have a slight negative affect on meat quality when fed for 30 d. If muscles from ZH fed cattle are negatively affected, it appears that they respond well to electrical stimulation and proper aging. Vasconcelos et al. (2008) found that cattle fed ZH for 20, 30 or 40 d, had increased (P < 0.01) HCW (4.5%), increased (P < 0.01) dressing percent (3.1 percentage points), less 12th rib fat (P < 0.01), larger (P < 0.01) ribeye areas (10.6%), and less KPH fat (P = 0.03) than control cattle. These authors suggested that

these results indicate a shift in how nutrients are partitioned between lean and fat depots.

Tenderness

With the use of β -agonists, it is apparent that the tenderness of meat can be adversely affected. The tenderness of beef is a major concern to most consumers. When consumers are asked which beef sensory attribute is most important in their individual assessments of overall satisfaction, most identify tenderness as their primary consideration (Platter et al. 2003). There are large inconsistencies due to variation in tenderness and β -agonists can add to this variation. There are several factors that are thought to have effects on tenderness, such as genetics, age, temperament, diet, time on feed, handling stress and metabolic modifiers (Choat et al., 2006). Effective preharvest management strategies can be applied to reduce variation in tenderness and minimize the variation in tenderness that stems from different management practices. There are also different postmortem strategies for improving tenderness of beef, such as postmortem aging and electrical stimulation. Tenderness can be measured by a trained sensory panel, a consumer panel, by blade tenderization or by taking instrumental shear force.

Aging

Postmortem aging of meat is probably the most widely used tenderizing process in the meat industry. Post mortem aging is used for periods of varying lengths from a few days to longer than a month (Savell et al., 1981). Aging of meat can be used to ensure a more tender product (Davey et al., 1967). Devine (2004) suggests that the process of aging involves the breakdown of muscle structural proteins by endogenous enzymes, such as calpains and cathepsins. Pringle et al. (1993) found that lambs treated with the

β-agonist, L₆₄₄,969 for 2 wks had 73% higher (p<0.01) calpastatin activity than control animals. The increased level remained higher throughout the duration of the study. These data indicated a decrease in proteolytic activity of animals fed the β-agonist. This likely is related to the decrease in tenderness and the decreased response to aging that can occur with β-agonist treatment (Pringle et al, 1993; Koohmaraie and Shackelford, 1991).

The benefits of aging can be affected by an animal's pre-slaughter history, or temperature and duration of aging. One of the most important factors affecting aging is the temperature of the meat. Meat will age rapidly at the start and more slowly over time. Aging will occur at different rates for different muscles and different species (Devine, 2004). However, higher temperatures can also facilitate higher bacterial growth and spoilage. Miller et al. (1997) determined that aging steaks for 14 d improved all sensory traits and Warner-Bratzler shear force values. They suggested that aging of beef for 14 d would improve the consistency of tenderness and should be recommended as a processing control point for the beef industry to improve consumer acceptance, regardless of the process the meat has gone through. The effect of aging generally is measured by tenderness of the meat before and after aging NCBA (2006).

One factor affecting the variability of aging time is muscle type. NCBA (2006) suggested that different muscles and different quality grades will have different aging responses. The aging response is the overall change in WBSF that occurs during aging. NCBA (2006) looked at numerous muscles and determined that Premium choice longissimus had a moderately high aging response (2.1 to 1.8 kg) and Select longissimus had a high aging response (≥ 2.2 kg). The Choice and Select gluteus medius had a moderate aging response (1.1 and 1.6 kg), and the Choice and Select triceps brachii also had a moderate aging response (1.4 and 1.6 kg).

Electrical stimulation

Electrical stimulation is another process that can be used to improve tenderness in meat and is widely accepted by U.S. beef processors due to its numerous benefits (Savell, 1979). According to Savell et al. (1978), steaks from non-electrically stimulated loins that were aged for 21 d had significantly higher shear force values than steaks from electrically stimulated loins that were aged for only 7 d. McKeith et al. (1981) conducted a study to show the effects of electrical stimulation on several major muscles of beef carcasses. The authors reported a decrease (P < 0.09) of shear force in the superficial pectoral, supraspinatus, subscapularis and longissimus dorsi muscles when electrically stimulated. Another study by Walker et al. (1977) showed a decrease in shear force of gluteus medius, vastus lateralis, biceps femoris and longissimus dorsi muscles when electrically stimulated. Savell (1979) reported that electrical stimulation appears to increase tenderness of meat if its initial tenderness would be unacceptable, but it does not appear to affect the tenderness of meat if its initial tenderness is acceptable. Electrical stimulation appears to accelerate the postmortem aging of beef as well (Savell et al. 1978). Electrical stimulation will rapidly drop the pH of carcasses, causing the fibers to enter rigor earlier at a higher temperature. These fibers that enter rigor at a higher temperature will have a rapid rate of aging (Devine, 2004).

Effects of gender on tenderness

One of the factors thought to influence tenderness of meat is gender classification (steer vs. heifers). Heifers typically have carcasses with higher marbling scores and more desirable USDA quality grades, but it is thought that steers typically produce more tender meat (Choat et al., 2006; Tatum et al., 2007). There are several factors associated with gender that have an effect on meat tenderness. Some of these factors are differences in calpastatin activity and its effect on early postmortem tenderness,

differences in temperament, stress levels and hormonal effects (Tatum et al., 2007). Wulf et al. (1996) found that longissimus tissue from heifers had higher calpastatin activity than steers. Higher calpastatin activity can lead to decreased activity of µ-calpain, which reduces the amount of post-mortem tenderization. Heifers and steers also have a difference in temperament. Heifers have been documented as being more excitable due to their increased estrogen secretions. This temperamental behavior of heifers has been shown to be significantly correlated with higher calpastatin activity, higher shear force values and lower sensory scores for tenderness and flavor (Wulf et al. 1997). Tatum et al. (2007) summarized 10 different studies that looked at the shear force values of longissimus steaks between steers and heifers. In eight out of 10 of the studies, shear force in heifers was significantly higher than for the steers. In the other two studies, no statistical difference was found. Heifers have also been shown to produce meat that is more variable in tenderness and, therefore, more likely to be unacceptable (Maher et al., 2004). Choat et al. (2006) conducted a study using steers and heifers to look at the differences in carcass characteristics and shear force. The steers were found to have heavier carcass weights and less 12th rib fat compared to the heifers. Before evaluating shear force, strip steaks from the steers and heifers were aged for 7, 14 or 21 d. When marbling was used as a covariate, shear force of the strip loin steaks from heifers were higher (P < 0.001) than steers after being aged for 7 d. After 14 d aging, steaks from the heifers still had a higher (P < 0.006) shear force than those from the steers. After 21 d of aging, tenderness of the steaks was not statistically different. Prost et al. (1975) conducted a study to determine the tenderness of beef among different muscles and genders. Differences (P < 0.05) in tenderness were reported among the different muscle types. Overall, shear forces did not show any differences (P > 0.05) between males and females, but there were some individual muscles, such as the semitendinosus that had a lower (P < 0.05) shear force for steers compared to heifers. The mean of all the muscles, however, showed higher (P < 0.05) sensory tenderness score for steers than heifers.

Conclusion

Beta-adrenergic agonists are potent growth promoters that are being used frequently in the livestock industry. They are used to increase efficiency of growth and improve carcass meat yield. These growth promoters achieve this increased meat carcass yield, by repartitioning nutrients away from fat deposition and towards muscle growth. Although, β -AA have this beneficial affect on muscle growth, they also cause a decrease in tenderness and potentially in intramuscular fat. Different lengths of feeding have been shown to increase or decrease these affects.

References

- Anderson P.T. 2000. Mechanisms by which metabolic modifiers alter growth rate and carcass composition of meat animals. Pages 31-35 in Proc. 53rd Recip. Meat Conf., Colombus, Ohio.
- Beerman, D. H., W. R. Butler, D. E. Hogue, V. K. Fishell, R. H. Dalrymple, C. A. Ricks and C. G. Scanes. 1987. Cimaterol-induced muscle hypertrophy and altered endocrine status in lambs. J. Anim. Sci. 65:1514-1524.
- Choat, W. T., J. A. Paterson, B. M. Rainey, M. C. King, G. C. Smith, K. E. Belk, and R. J. Lipsey. 2006. The effects of cattle sex on carcass characteristics and longissimus muscle palatability. J. Anim. Sci. 84:1820-1826.
- Davey, C. L., H. Kuttel and K. V. Gilbert. 1967. Shortening as a factor of meat aging. Food Technol. 2:53-55.
- Devine, C. E. 2004. Aging. Pages 330-338 in Encyclopedia of Meat Sciences. W. K. Jensen, C. Devine, and M. Dikeman, eds. Elsevier, Ltd., Oxford, UK.
- Dikeman, M. E. 2000. Effects of metabolic modifiers used in animal production on meat quality. Pages 36-56 in Proc. 53rd Recip. Meat Conf.
- Dikeman, M. E. 2003. Metabolic modifiers and genetics: Effects on carcass traits and meat quality. Brazilian journal of food technology (special issue) 6:1-38.

- Kim, Y. S., and R. D. Sainz. 1992. B-adrenergic agonists and hypertrophy of skeletal muscles. Life Sci. 50:397-407.
- Koohmaraie M. and S. D. Shackelford. 1991. Effect of calcium chloride infusion on the tenderness of lambs fed a beta-adrenergic agonist. J. Anim. Sci. 69:2463-2471.
- Maher S. C., A. M. Mullen, A. P. Moloney, D. J. Buckley and J. P. Kerry. 2004.

 Quantifying the extent of variation in the eating quality traits of the M. longissimus dorsi of conventionally processed Irish beef. Meat Sci. 66:351-360.
- McElligott, M.A., A. J. Barreto, and L. Y. Chaung. 1989. Effect of continuous and intermittent clenbuterol feeding on rat growth rate and muscle. Comp. Biochem. Physiol. 92C:135–138.
- McKeith, F. K., J. W. Savell and G. C. Smith. 1981. Tenderness improvement of the major muscles of the beef carcass by electrical stimulation. J. Food. Sci. 46:1774-1776.
- Mersmann, H. J. 1998. Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. 76:160-172.
- Miller, M. F., C. R. Kerth, J. W. Wise, J. L. Lansdell, J. E. Stowell, and C. B. Ramsey. 1997. Slaughter plant location, USDA quality grade, external fat thickness, and aging time effects on sensory characteristics of beef strip loin steak. J. Anim. Sci. 75:662-667.

- Moloney, A., P. Allen, R. Joseph, and V. Tarrant. 1991. Influence of beta-adrenergic agonists and similar compounds on growth. In: A. M. Pearson, and T. R. Dutson (Ed.) Growth Regulation in Farm Animals. Advances in Meat Research (Vol. 7) pp 455-513. Elsevier Applied Science, New York.
- NCBA (National Cattlemen's Beef Association) 2006. Pages 1-10 in Industry guide for beef aging. National Cattlemen's Beef Association. Englewood, CO.
- Plascenia A., N. Torrentera and R. A. Zinn. 1999. Influence of the beta-agonist zilpaterol, on growth performance and carcass characteristics of feedlot steers. J. Anim. Sci. 77(Suppl. 1):114.
- Platter W. J., W. T. Choat, W. J. Platter. 2008. Balancing beef quality and red meat yield with ractopamine hydrochloride. Pages 1-6 in Proc. 61st Recip. Meat Conf., Gainesville, Florida.
- Platter, W. J., J. D. Tatum, K. E. Belk, P. L. Chapman, J. A. Scanga and G. C. Smith. 2003. Relationships of consumer sensory ratings, marbling score and shear force value to consumer acceptance of beef strip loin steaks. J. Anim. Sci. 81:2741-2750
- Pringle, T.D., C. R. Calkins, M. Koohmaraie, and S. J. Jones. 1993. Effects over time of feeding a β-agonist to wether lambs on animal performance, muscle growth, endogenous muscle proteinase activities, and meat tenderness. J. Anim. Sci. 71:636-644.

- Prost, E., E. Pelczynska and A. W. Kotula. 1975. Quality characteristics of bovine meat.

 II. Beef tenderness in relation to individual muscles, age and sex of animals and carcass quality grade. J. Anim. Sci. 41:541-547.
- Savell, J. W. 1979. Update:industry acceptance of electrical stimulation. Pages 113-115 in Proc. 32nd Recip. Meat Conf., Brookings, South Dakota.
- Savell J. W., G. C. Smith and Z. L. Carpenter. 1978. Beef quality and palatability as affected by electrical stimulation and cooler aging. J. Food. Sci. 43:1666-1668.
- Schiavetta A. M., M. F Miller, D. K. Lunt, S. K. Davis and S. B. Smith. 1990. Adipose tissue cellularity and muscle growth in young steers fed the β-adrenergic agonist clenbuterol for 50 days and after 78 days of withdrawal. J. Anim. Sci. 68:3614–3623.
- Sillence, M. N. and M. L. Matthews. 1994. Classical and atypical binding sites for β-adrenoceptor ligands and activation of adenylyl cyclase in bovine skeletal muscle and adipose tissue membranes. Brit. J. Pharm. 111: 866–872.
- Strydom, P. E., L. Frylinck, and G. L. Marais. Supplemental vitamin D3 and electrical stimulation to reduce the effect of beta agonists on meat quality. 2007. Pages 273-274 in Proc. 53rd Int. Congr. Meat Sci. Technol., Beijing, China.

- Strydom, P. E., and E. Nel. 1999. The effect of supplementation period of a beta-agonist (zilpaterol), electrical stimulation, and aging period on meat quality characteristics.

 Pages 58-59 in Proc. 45th Int. Congr. Meat Sci. Technol., Yokohama, Japan.
- Tatum J. D., S. L. Gruber and B. A. Schneider. 2007. Pre-harvest factors affecting beef tenderness in heifers. Pages 1-18 in executive summary prepared for NCBA. Centennial, CO
- Vasconcelos J. T., R. J. Rathmann, R. R. Reuter, J. Leibovich, J. P. McMeniman, K. E. Hales, T. L. Covey, M. F. Miller, W. T. Nichols and M. L. Galyean. Effects of duration of zilpaterol hydrochloride feeding and days on the finishing diet on feedlot cattle performance and carcass traits. J. Anim Sci. 1910. doi:10.2527/jas.2008-1032
- Walker, D. J., P. V. Harris and F. D. Shaw. 1977. Accelerated processing of beef. J. Food Technol. Austral. 29:504-506.
- Wulf D. M., J. D. Tatum, R. D. Green, J. B. Morgan, B. L. Golden and G. C. Smith. 1996.
 Genetic influences on beef longissimus palatability in charloais and limosine sired steers and heifers. J Anim. Sci. 74:2394-2405.
- Yang, Y. T. and M. A. Mc Elligott. 1989. Multiple actions of β-adrenergic agonists on skeletal muscle and adipose tissue. Biochem. J. 261:1-10.

CHAPTER 2 - Effects of supplementing feedlot steers and heifers with zilpaterol hydrochloride on Warner Bratzler Shear Force of steer Longissimus lumborum and Heifer Longissimus lumborum, Triceps brachii and Gluteus medius muscles aged for 7, 14 and 21 d.

Introduction

To meet increasing demands for meat and to maximize efficiency, beef producers have begun to use new management strategies, such as the use of a class of feed additives called beta-adrenergic agonists. Mexico and South Africa approved the use of the beta-adrenergic agonists ractopamine-HCL (Optaflexx[™]) and zilpaterol-HCL (Zilmax®; ZH) more than 10 yr ago. In 2006, ZH was approved for use in the United States (Avendano-Reyes et al., 2006).

Zilpaterol has been found to increase rate of gain, increase dressing percent, improve feed efficiency and improve carcass composition. This compound acts by repartitioning nutrients away from fat gain and towards increased muscle growth (Beerman, 2004). Although beta-agonists can increase muscle mass, they have also been shown to decrease meat tenderness. In a study conducted by Wheeler and Koohmarie (1992), steers were fed the beta-agonist $L_{644,969}$ for 1, 3, 5 and 6 wk. Muscle calpastatin levels, which inhibit the calpain proteolytic system, and tenderness were measured. Calpastatin levels were higher (p < 0.05) in beta-agonist fed steers than controls, and tenderness was decreased (p < 0.05) in beta-agonist fed animals.

It has long been known that postmortem aging of muscle will increase tenderness. According to Miller et al. (1997), beef strip steaks aged for 14 d had

decreased (p < 0.05) WBSF values when compared to steaks aged for 7 d. Davey et al.(1967); Devine (2004) and Savell et al. (1981) also suggested that aging of meat can be used to ensure a more tender product.

Since just recently being approved for use in the US, there have been few studies on feeding ZH to both steers and heifers for different lengths of time using different aging periods. Therefore, research needed to be done on how different treatments of ZH effect tenderness and important carcass characteristics, such as ribeye area, adjusted preliminary yield grade, and percent chemical fat of different muscles.

Therefore, the objective of this study was to determine the effects of feeding ZH to both steers and heifers for three different lengths of time on tenderness of three different muscles, using three different aging times. With the data collected, it was hoped that an optimal length of time to feed ZH along with an optimal aging time could be determined in order to minimize the toughening effect of ZH while still benefiting from its effects on performance and carcass composition.

Materials and Methods

Animal Treatments and Experimental Design

One hundred and seventeen steers and 132 heifers were used for this study. Both genders were fed at Cactus Research, Cactus, TX. Both steers and heifers were British and British x Continental crossbreds with average initial weights of 333 kg (steers) and 318 kg (heifers). All cattle were vaccinated, dewormed and fed the same base diet (Appendix A). Both steers and heifers were blocked by initial weight into 6 blocks. There were 24 different pens for both steers and heifers. There were six blocks of four pens and each pen contained one treatment. Zilmax® (ZH) treatment was fed at a

concentration of 7.56 g/907kg on a 100% DM basis daily. One pen in each block received no ZH (Zilmax®, Intervet, DE), one received ZH for 20 d, one received ZH for 30 d and the last pen received ZH for 40 d. All animals ended their treatment 3 d prior to slaughter to allow for a withdrawal period. Steers were slaughtered in two groups at Tyson Fresh Meats, Amarillo, TX and all heifers were slaughtered at a later date in two groups at Tyson Fresh Meats, Amarillo, TX. The first half of each gender was slaughtered at a weight acceptable to a typical feed yard and by visual appraisal of finish, with the target of approximately 60% Choice and a maximum of 15% yield grade 4 carcasses.

Muscles

Steer strip loins (NAMP #180) and heifer strip loins, shoulder clods (NAMP #114) and center-cut top sirloins (NAMP #184D) were received at the Kansas State University Meat Laboratory from (Tyson Fresh Meats, Amarillo, TX) at 7 d postmortem. The muscles were received in four different shipments according to slaughter date. The first two shipments contained 54 and 63 steer strip loins, respectively. The third shipment contained 72 heifer strip loins, 59 heifer shoulder clods and 59 heifer center-cut top sirloins. The fourth shipment contained 60 heifer strip loins, 59 heifer shoulder clods and 59 heifer center-cut top sirloins.

Fabrication and Aging

Strip Loin. On all four shipment days, strip loins were weighed, removed from their vacuum package, blotted and weighed again. The strip loins then had to be cut into 9 uniform, 2.54-cm thick steaks. Each strip loin was first faced to square the anterior end

of the subprimal and this section was used for proximate analysis. Three of the steaks were used for Warner-Bratzler shear force (WBSF) determinations and the remaining six were sent to the Texas Tech University Meats Laboratory for consumer sensory analysis. For the first strip loin, steak # 1 was used for 7 d aging and WBSF determinations, steak # 2 was used for 14 d aging and steak # 3 was used for 21 d aging. For each loin after this, the numbers were rotated so that steak # 2 of the second loin was used for 7 d aging; steak # 3 of the third loin was used for 7 d aging etc., which resulted in each location represented an equal number of times.

All steaks were labeled and vacuum packaged individually (62.2 cm Hg vac; Multivac C500; Multivac, Inc., Kansas City, MO). The proximate analysis steaks and the steaks that were to be aged for 7 d were frozen immediately in a (-40 °C) blast freezer. After frozen, the steaks were moved to another freezer (-29 °C) until analysis. The steaks that were to be used for 14-d WBSF determinations were placed in a cooler (2°C) for an additional 7 d before freezing and the steaks to be used for 21-d WBSF were placed in the cooler for an additional 14 d before freezing. After proper aging time, these steaks were also blast frozen (-40 °C), then held in the other freezer (-29 °C) until analysis.

Shoulder Clod. On the last two shipment days, heifer shoulder clods were weighed, removed from their vacuum package, blotted and weighed again. The clod heart (NAMP# 114E) was trimmed and the triceps brachii long head was removed. The triceps brachii long head was faced on the ventral end to square it, and this section was used for proximate analysis. The triceps brachii long head was then fabricated into three uniform, 2.54cm thick steaks for 7, 14 and 21 d WBSF determinations.

Steaks were vacuum packaged and labeled using the same method as for the strip loins, with the first steak from the first triceps aged for 7 d and the second steak

from the second triceps aged for 7 d. The steaks were aged for the appropriate number of days and blast frozen using the same method as for the loin steaks.

Top Sirloin. On the last two shipment days, heifer top sirloins were removed from their vacuum packages and weighed using the same procedure as with the strip loins and shoulder clods. Next, the biceps femoris, gluteus accesorius and gluteus profundus were removed. The GM was then faced to square the anterior end, and this section was used for proximate analysis. The remaining GM was cut into three uniform, 2.54 cm thick steaks for 7, 14 and 21 d aged WBSF determinations. Steaks were packaged, labeled, aged and frozen using the same method as for the loin and triceps steaks.

Cooking

Longissimus lumborum, Triceps brachii and Gluteus medius steaks. Steaks were in frozen storage for 2-4 mo depending on the date of fabrication. Steaks were thawed and cooked in the order that they were fabricated. Only one muscle type was cooked per day. Steer Longissimus lumborum (LL) steaks were cooked first, heifer LL were cooked second and heifer Triceps brachii (TB) and Gluteus medius (GM) steaks were cooked last. Between 30 and 120 steaks were cooked and sheared each time; approximately one third were 7 d, one third 14 d and one third 21 d aged. All steaks were cooked and sheared within 8 wk.

Steaks were taken out of the freezer, placed in a single layer on a metal tray, and placed in a refrigerator (2 °C) for 24 hr to thaw. Steaks were removed from their vacuum packages, blotted with a paper towel and weighed before cooking. The temperature of each steak was taken to ensure that they were between 0 and 4°C. Steaks were cooked on a fully warmed "Next Grilleration" George Foreman Digital Grill (Model GRP99) to a

medium degree of doneness (~71°C). Internal temperature was monitored using a 30-gauge, copper-constantan type T thermocouple inserted into the geometric center of each steak and attached to a Doric temperature recorder (model 205; Vas Engineering, San Francisco, CA). After cooking, steaks were immediately weighed and percent cook losses were calculated. Steaks were then placed on a metal tray with poly vinyl overwrap to prevent moisture loss and placed in a refrigerator (2°C) for 24 hr so the steaks would reach an ultimate temperature of 2 to 5°C before coring.

Warner-Bratzler Shear Force Determinations

Cooked LL, TB and GM steaks were removed from refrigerator storage and cores were collected from each steak. The cores were removed parallel to the muscle fiber orientation, taking care to avoid heavy connective tissue and fat. Six cores were taken in total; two from the lateral, middle and medial portions, respectively. Each core was sheared once using a WBS Testing Machine (G-R Elec. Mfg. Co., Manhattan, KS). The machine was set to zero and the shear blade was cleaned before each shear. The shear force of each core was recorded in (kg) and entered into an excel sheet, where the average of the six shears was taken.

Statistical Design

There were 117 steers and 132 heifers in this study. The experimental design for the effect of ZH treatment on WBSF was a randomized complete block design with a split-plot. There were 6 blocks containing 4 pens each. The blocking factor was initial animal weight and each pen contained one treatment. The whole-plot treatment factor was ZH at feeding levels of 0, 20, 30 and 40 d. The animal was the subsample unit on the whole-plot. The split-plot treatment factor was days of aging at three levels (7, 14,

and 21 d), applied to a muscle (steer) or muscles (heifer) from each animal. The wholeplot experimental unit was the pen, the subsample experimental unit on the whole-plot was the animal, and the split-plot experimental unit was the steak. All heifer and steer data were analyzed separately.

The experimental design for the effect of treatment on % IMF was a randomized complete block design, with animals represented as subsampling within the experimental unit (pen). This was the same as for the effect of ZH treatment on WBSF, except there was no split-plot and no aging factor.

The design for using % IMF as a covariate was the same as for the effect of ZH treatment on WBSF, except that % IMF was added as a covariate on the whole-plot.

These three procedures were done using the MIXED procedure in SAS (SAS Institute, inc., Cary, NC). Subsets of least squares means were subjected to pairwise comparisons with Tukey-Kramer adjustments. The main effects of treatment and aging and their interactions were tested using a p-value of .05.

The design for testing a threshold level of WBSF was the same as for testing the effect of ZH treatment on WBSF. The procedure was done using GENMOD with binomial distribution and the logit link function.

The design to calculate correlations was a randomized complete block design, which was the same design used for testing the effect of ZH treatment of WBSF. A multivariate analysis of variance (MANOVA) was used to get partial correlation coefficients.

Results and Discussion

To date, there are few published studies on feeding ZH to cattle. Most of the studies on feeding beta-agonists used ractopamine (Beerman, 2004; Avendano-Reyes

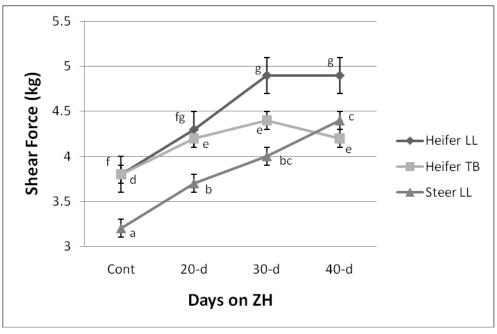
et al., 2006), clenbuterol (Schiavetta et al., 1990), or L644-969 (Pringle et al., 1993). Most previous studies that looked at ZH fed for 30, 40 or 50 d and only looked at the longissimus muscle (Beerman, 2004; Plascenia et al. 1999; Strydom and Nel, 1999; Avendano-Reyes et al., 2006). Beerman (2004) showed an increase in muscle growth rate and a reduced amount of fat with the feeding of ractopamine. Schiavetta et al. (1990) showed that marbling scores were decreased (P < 0.05) and shear force values were increased 19% (P < 0.05) in steers fed clenbuterol for 50 d. Pringle et al. (1993) showed that longissimus shear force values were higher (P < 0.001) in lambs fed L644-969 for 2, 4 and 6 weeks than controls. Some of the studies on feeding ZH to cattle were limited to investigating its effects on carcass characteristics, where ZH fed cattle had heavier HCW (P < 0.01), greater dressing percent (P < 0.01) and larger LM area (P < 0.01) (Vasconcelos et al.,2008; Plascenia et al., 1999). Few studies have looked at the effect of ZH treatment on tenderness using WBSF. Most of the studies on feeding beta-agonists have been on steers and have not looked at the effects of beta-agonists on heifers.

Effect of treatment on WBSF

Steer LL. There was no (P > 0.05) treatment by aging interaction for steer LL muscle WBSF. Therefore, ZH treatment and aging means are shown in Figures 1 and 3, respectively. Feeding ZH at 7.5g/907kg to steers for 20 d increased (P < 0.05) WBSF of LL steaks .5 kg compared with controls (C) (Figure 1). Longissimus lumborum steaks from steers fed no ZH had the lowest (P < 0.05) WBSF compared with ZH fed treatments. Steaks from steers fed ZH for 20 d had 0.08 kg lower (P < 0.01) WBSF than steaks from steers fed ZH for 40 d. These results agree with those of Avedano-Reyes et al. (2006), who found that administration of ZH (60 mg/steer/day) for 33 d increased (P < 0.01) WBSF of LL muscle compared with C.

Research has been published on values of WBSF that might correspond to unacceptable tenderness to consumers (Miller et al., 2001; Platter et al., 2003 and Lorenzen et al., 2003). Platter et al. (2003) reported values of 4.4 kg as being the threshold for unacceptability. Miller et al. (2001) reported that WBSF values of 3.0, 3.4, 4.0, 4.3 and >4.9 kg resulted in consumer acceptance ratings of 100, 99, 94, 86 and 25%. For my study, we used 5 kg as a threshold of acceptability. This value is probably on the liberal side according to other studies, but could be interpreted that the portion of the steaks > 5 kg would clearly be unacceptable to consumers. Our mean WBSF values for the 20 and 30 d treatments are in an acceptable range, but 24% of the steaks from the 40 d treatment were clearly unacceptable and had WBSF values ≥ 5 kg.

Figure 1. Effects of Zilpaterol treatment on Warner-Bratzler shear force of steer longissimus lumborum, heifer longissimus lumborum and heifer triceps brachii muscle



Differences within the steer LL muscle with different superscript letters differ (P < 0.05)

de Differences within the heifer TB muscle with different superscript letters differ (P < 0.05)

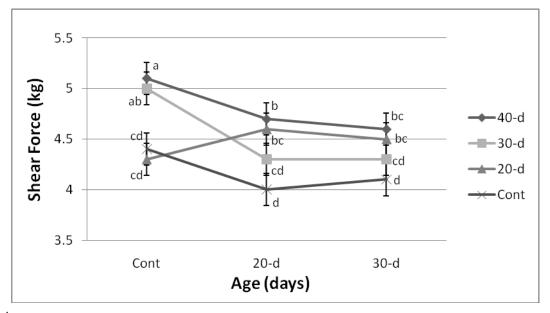
 $^{^{}m fg}$ Differences within the heifer LL muscle with different superscript letters differ (P < 0.05)

Heifer LL and TB muscles. There was no treatment by aging interaction (P > 0.05) for WBSF of heifer LL or TB muscles. The ZH treatment and aging means on heifer LL and TB muscles are shown in figures 1 and 4, respectively. Longissimus steaks from heifers fed no ZH had (>1.1kg) lower (P < 0.05) WBSF than steaks from heifers fed ZH for 30 or 40 d (Figure 1). Using ≥ 5 kg WBSF as a threshold for unacceptable tenderness, 29% and 33% of the 30 d and 40 d treatments, respectively, were unacceptable.

Triceps brachii steaks from heifers fed ZH for 20, 30 and 40 d had an increased (P < 0.05) WBSF over C; however, there were no (P < 0.05) differences among the 20, 30 and 40 d ZH treatments (Figure 1).

Heifer GM interaction. There was a treatment by aging interaction (P < 0.05) for WBSF of heifer GM muscle (Figure 2). Means for the 30 d and 40 d treatments decreased (P < 0.05) from 7 to 14 d but no treatment decreased from 14 to 21 d. Means for the 30 and 40 d treatments were higher than C and the 20 d treatment after 7 d of aging and higher than C after 14 and 21 d of aging. The mean for the 40 d treatment were higher than the 30 d treatment after 14 d of aging.

Figure 2. Interaction between aging and Zilpaterol treatment on Warner-Bratzler shear force of heifer Gluteus medius muscle



abcd
Differences among ZH treatment by aging means with different superscript letters differ (P < 0.05)

The WBSF of GM muscles from heifers treated with ZH for 20 d were not different (P > 0.05) from C after 7 d of aging, or from the 30 or 40 d treatments after 14 and 21 d of aging. After 21 d of aging, WBSF of GM muscles from heifers treated with ZH for 40 d were higher (P < 0.05) than C by .3 and .6 kg respectively. There was no difference (P > 0.05) in WBSF of muscles between heifers treated with 30 and 40 d ZH at 7 d of aging, but there were differences after only 14 d of aging (Figure 2).

Strydom and Nel (1999) stated that the effects of ZH on meat quality was muscle specific. Strydom et al.(2007), Strydom and Nel (1999), Beerman (2004) and Avendano-Reyes et al. (2006) found negative effects of ZH on steer or bull LL muscle. Our study shows that along with steers, there are also negative effects on tenderness of muscles from heifers fed ZH. My study also shows that, along with the LL muscle, the TB and GM are also impacted negatively.

Effect of aging on WBSF

Steer LL. As the aging time of steer LL muscle increased from 7 to 14 and 21 d, WBSF decreased (P < 0.01) in a linear fashion from 4.1 to 3.8 to 3.5 kg (Figure 3). These results are consistent with those of NCBA (2006) that steer LL muscle decreased in WBSF from 7 to 14 d of aging; however, the difference I found was less than the 1 kg in that publication.

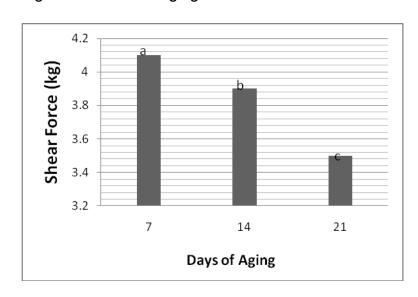


Figure 3. Effects of aging on WBSF of steer LL muscle

Heifer LL and TB. As the aging of heifer LL muscle increased from 7 to 14 and 21 d, WBSF decreased (P < 0.01) from 5.2 to 4.3 and 3.9 kg, respectively (Figure 4). The WBSF of heifer TB muscle decreased (P < 0.01) to a lesser extent from 4.4 to 4.1 to 3.9 kg for 7, 14 and 21 d aging, respectively. These results and those for the steers, agree with those of Miller et al. (1997) who determined that aging steaks for 14 d improved WBSF values over those aged for 7 d. Devine (2004) suggested that aging of meat is an exponential process that is rapid at the start, then slows over time. My results agree with this because the heifer LL and TB muscle appear to increase in tenderness at

a more rapid rate from 7 to 14 days of aging, then slow from 14 to 21 days of aging. Devine (2004) and NCBA (2006), also suggested that different muscles age at different rates. In my study the heifer LL appears to age at a more rapid rate than the TB (Figure 4) and the GM (Figure 2). This finding also agrees with NCBA (2006), that the aging of longissimus muscle occurs at a more rapid rate than GM or TB muscle.

5.5

Shear Formula (kg)

4.5

a

elimination of aging

7

14

Days of aging

21

Days of aging

Figure 4. The effect of different aging times on Warner-Bratzler shear force of heifer Longissimus lumborum and Triceps brachii muscles

Response to Aging

Steer LL. There was no treatment by aging interaction (P > 0.05) for steer LL muscle, so the main effect of aging steer LL muscle is shown in figure (3). There was a greater reduction in WBSF from 14 to 21 d than from 7 to 14 d. After aging steer LL for 21 d, there was only 3.7% of steaks that had a WBSF value of \geq 5 kg for the 40 d

 $^{^{}m abc}$ Differences within the heifer TB muscle with different superscript letters differ (P < 0.05) $^{
m def}$ Differences within the heifer LL muscle with different superscript letters differ (P < 0.05)

treatment of ZH, indicating that 21 d of aging appears to be effective for making tenderness of steaks acceptable from steers fed ZH for 40 d.

Pringle et al. (1993) reported a decreased response to aging from 2 to 7 d in L_{644,969} treated lambs. Koohmaraie and Shackelford (1991) also reported a decreased response to aging from 1 to 14 d in lambs fed the beta-agonist L_{644,969}. Our results generally disagree with the findings of these studies. Our results may have differed due either to species differences or to the overall length of aging in our study, which was longer than these. From 7 to 14 d, the aging response was not adequate to make LL steaks from cattle fed ZH for 40 d acceptagble in tenderness. After 14 d, the aging process appeared to be more effective for reducing WBSF.

Heifer LL, TB and GM. All three muscles from the 40 d treatment of ZH had the greatest response to aging, whereas C had the least (Figure 4). For heifer GM muscles, the 30 d treatment generally had the greatest response to aging, but 20 d had no response (Figure 2). After aging heifer LL steaks for 21 d, 6.6% of the steaks still had WBSF ≥ 5kg. For heifer TB muscles aged for 21 d from heifers fed ZH for 40 d, only 1.3% had a WBSF ≥ 5 kg. For heifer GM muscle aged 21 d from the 40 d treatment, 10.7% of the steaks still had a WBSF ≥ 5 kg. When just looking at means in graphs of WBSF of different muscles after aging and treatment, the means may look acceptable, but in the case of the heifer GM, several of the steaks likely would be rated unacceptable by consumers. Our results for the effects of aging on heifer LL and TB disagree with the results of Pringle et al. (1993) and Koohmaraie and Shackleford (1991), who indicate that muscles from beta-agonist treated animals respond less favorably to aging.

Effect of treatment on percent intramuscular fat

Steer LL/ Heifer LL, TB and GM. There were no differences (P > 0.05) in % IMF of steer LL, heifer LL, heifer TB or heifer GM muscle among 0, 20, 30 or 40 d treatments of ZH. However, the % IMF of steer LL muscle did have a trend (P=0.06) of decreasing as ZH treatment increased from C to 40 d (data not shown). Gruber et al. (2007) and Walker et al. (2006) determined that steers and heifers fed the beta-agonist ractopamine, had decreased marbling scores. My results from feeding ZH did not agree with the results of these studies. These differences may be due to the fact that my study measured ether extractable % IMF and the others used a marbling score. Plascencia et al. (1999) also found that feeding ZH to cattle did not affect marbling score. Quinn et al. (2008) found results similar to mine, that no treatment differences (P > 0.31) were found for marbling scores when feeding heifers ractopamine.

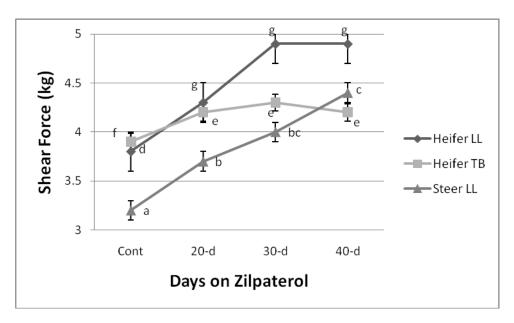
Steer LL covariate analysis. When % IMF was used as a covariate in testing for differences among ZH treatments and aging, there was no (P > 0.05) ZH treatment by aging interaction for steer LL muscle using percent fat as a covariate; which is the same as when not using % IMF in the analysis of variance (Figures 1 and 5). The main effects from the covariate analysis are shown in figure (5).

Heifer LL, TB and GM covariate analysis. There were no treatment by aging interactions (P > 0.05) for heifer LL or TB muscle when using % IMF as a covariate which is the same as when not using % IMF in the analysis for variance (Figures 1 vs 5). The main effects of treatment using % IMF as a covariate are shown in (Figure 5).

There was a treatment by aging interaction (P < 0.05) for heifer GM muscle when using % IMF as a covariate (Figure 6). This is similar to the effect of ZH treatment and aging on heifer GM muscle without using the covariate. Therefore, it did not matter

whether I used % IMF as a covariate, the effects of ZH treatment and aging on the GM were the same.

Figure 5. Effects of Zilpaterol treatment on Warner-Bratzler shear force of steer Longissimus lumborum, heifer Longissimus lumborum and heifer Triceps brachii muscle using % IMF as a covariate

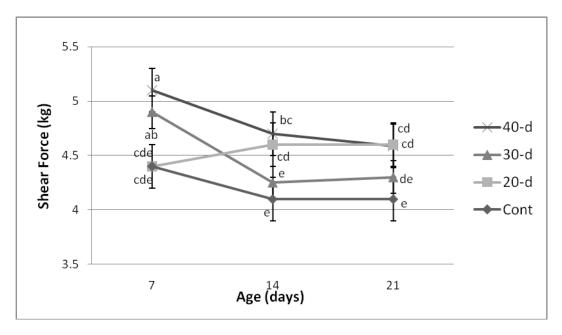


 $^{^{}abc}$ Differences within the steer LL msucle with different superscript letters differ (P < 0.05)

 $^{^{}fg}$ Differences within the heifer LL muscle with different superscript letters differ (P < 0.05)

 $^{^{}m de}$ Differences within the heifer TB muscle with different superscript letters differ (P < 0.05)

Figure 6. Effects of feeding Zilpaterol and aging on heifer Gluteus medius muscle using % intramuscular fat as a covariate



 $^{
m abde}$ Differences among ZH treatment by aging means with different superscript letters differ (P < 0.05)

Based on the comparison of analysis of variance and analysis of covariance, the effects of intramuscular fat on tenderness appear to be minimal. These results might have been expected because ZH treatment was not found to have any significant effects on % IMF of any muscle. Although there are only slight differences, it may still be more accurate to use a covariate analysis when looking at effects of ZH treatment on WBSF because % IMF can have some affect on shear force.

When using % IMF as a covariate and looking at the effects of aging on WBSF, the same results were found when keeping % IMF in the equation. This indicates that % IMF did not affect the tenderness of the steer LL or heifer LL, TB and GM muscle in my study.

Correlations among % IMF, ZH treatment, aging time, and WBSF

Steer LL. The WBSF values among the three aging times are presented in Table 1. It is interesting that all correlations were significant (*P* < 0.05), except for 7-d WBSF within the 30-d ZH treatment. We have no explanation for this deviation, although it might be that for the 40-d treatment, marbling is important for buffering the toughening effect of this treatment. In contrast, 14-d WBSF was consistently correlated with 21-d WBSF, regardless of ZH treatment. In addition, % IMF tended (*P* > 0.05) to be negatively correlated with 7- and 14-d WBSF for C and 7- and 21-d WBSF for the 40 d treatment.

Table 1. Correlations among %IMF and Warner-Bratzler shear force of steer LL at 7, 14 and 21 d postmortem with 0, 20, 30 and 40 d Zilmax®

					T	reatments1							
		WBSF, 7 d				WBSF, 14 d					WBSF, 21 d		
	С	20 d	30 d	40 d	C	20 d	30 d	40 d	С	20 d	30 d	40 d	
%IMF, C	-0.46				-0.46				-0.22				
%IMF 20 d		-0.13				-0.12				-0.38			
%IMF 30 d			-0.18				-0.25				-0.42		
%IMF 40 d				-0.44				-0.30				-0.45	
WBSF, 7 d					0.78**	0.83**	0.21	0.68**	0.68**	0.68**	0.26	0.83**	
WBSF, 14 d									0.84**	0.75**	0.60**	0 .71**	

 $^{^{1} ** =} P < 0.01; * = P < 0.05.$

Table 2. Correlations among %IMF and Warner-Bratzler shear force of heifer LL at 7, 14 and 21 d postmortem with 0, 20, 30 and 40 d Zilmax® treatments

						Treatments ¹						
		WBSF, 7 d				1	WBSF, 14 d		WBSF, 21 d			
	С	20 d	30 d	40 d	С	20 d	30 d	40 d	C	20 d	30 d	40 d
%IMF, C	-0.48**				-0.34				-0.48**			
%IMF, 20 d		-0.45**				-0.33				-0.25		
%IMF, 30 d			-0.50*				-0.36				-0.47**	
%IMF, 40 d				-0.13				-0.13				0.09
WBSF, 7 d					0.88**	0.92**	0.78**	0.74**	0.90**	0.83**	0.66	0.60**
WBSF, 14 d									0.93**	0.81**	0.73**	0.76**

^{1** =} P < 0.01; * = P < 0.05.

The % IMF of C heifer LL muscle was negatively correlated (P < 0.05) with WBSF of LL muscle aged for 7 and 14 d (Table 2), although the correlations were not especially high. The WBSF among the three aging times were all highly correlated (P < 0.01) and the magnitudes of the correlations were slightly higher than for steers. The % IMF of 20 and 30-d heifer LL muscle was negatively correlated (P < 0.05) with 7- and 21-d WBSF, respectively.

The % IMF of C heifer TB muscle was negatively correlated (P < 0.05) with WBSF of TB muscle aged for 7, 14 and 21 d (Table 3). These correlations were somewhat higher than those of the LL muscle. Low relationships among % IMF within the ZH treatments might be because the TB muscle has a low % IMF and, when cattle were treated with ZH, the percent was not altered enough to have an impact on WBSF.

WBSF of muscle aged for 14 d was also positively but moderately correlated (P < 0.05) with WBSF of muscle aged for 21 for the C and 20-d treatments only. However,

WBSF at 7 d was not (P > 0.10) correlated with WBSF at 21 d within the 30- and 40-d treatments.

Table 3. Correlations among % IMF and Warner-Bratzler shear force of heifer triceps brachii at 7, 14 and 21 d postmortem with 0, 20, 30 and 40 d Zilmax

					Tr	eatments ¹							
		WBSF, 7 d				WBSF, 14 d				WBSF, 21 d			
	С	20 d	30 d	40 d	С	20 d	30 d	40 d	С	20 d	30 d	40 d	
%IMF, C	41*				52**				-0.57**				
%IMF, 20 d		-0.33				-0.26				-0.24			
%IMF, 30 d			-0.29				-0.36*				0.07		
%IMF, 40 d				0.12				-0.10				-0.18	
WBSF, 7 d					0.59**	0.63**	0.56**	0.54*	0.64**	0.55**	0.23	0.24	
WBSF, 14d									0.40*	0.55**	0.04	0.44	

 $^{^{1}** =} P < 0.01; * = P < 0.05.$

Heifer GM. Correlations for the GM muscles (Table 4) were distinctly lower than for the other muscles. Except for the significant correlation between % IMF within the 30-d treatment and 30-d WBSF, none of the correlations for % IMF were significant (*P* > 0.10). Correlations of 7-d WBSF with 14-d WBSF were significant (*P* < 0.05), but not high. The 7-d WBSF was highly correlated with 21-d WBSF for the 40-d treatment only. Correlations of 14-d WBSF with 21-d WBSF were quite low within the 20-, 30- and 40-d ZH treatments.

Table 4. Correlations among %IMF and Warner-Bratzler shear force of heifer gluteus medius muscle with Warner-Bratzler shear force at 7, 14 and 21 d postmortem with 0, 20, 30 and 40 d Zilmax®

							Treatme	ents ¹						
		WBSF, 7 d				WBSF, 14 d					WBSF, 21 d			
	С	20 d	30 d	40 d	С	20 d	30 d	40 d	С	20 d	30 d	40 d		
%IMF, C	0.05				.12				-0.21					
%IMF, 20 d		-0.22				-0.36				-0.09				
%IMF, 30 d			-0.21				-0.38*				0.04			
%IMF, 40 d				0.31				-0.26				0.15		
WBSF, 7 d					0.50**	0.49**	0.43*	0.48*	0.35	0.26	0.24	0.70**		
WBSF, 14 d									0.46*	0.35	0.23	0.21		

 $^{^{1}}$ ** = P < 0.01; * = P < 0.05.

Heifer LL, TB and GM Correlations

Correlations among WBSF of different muscles for different aging times are presented in Tables 5 through 8. Correlations among the muscle by aging treatment combinations were quite low, except for moderate (P < 0.01) correlations between 7- and 14-d WBSF for both the TB and GM muscles. These results suggest that if WBSF increases or decreases for the LL, TB or GM muscles, this might not have an effect on the WBSF of other muscles from the same animal aged for the same times.

Table 5. Correlations among Warner-Bratzler shear force of control heifer LL, triceps brachii and gluteus medius muscle aged for 7, 14 and 21 d

Column1	LLWBSF14	LLWBSF21	TBWBSF7	TBWBSF14	TBWBSF21	GMWBSF7	GMWBSF14	GMWBSF21
LLWBSF7	0.84**	0.83**	-0.03	-0.15	-0.09	0.11	0.24	0.08
LLWBSF14	1	0.84**	0.02	-0.16	0.002	0.16	0.17	0.07
LLWBSF21		1	0.21	-0.03	-0.07	0.1	0.37	0.07
TBWBSF7			1	0.55**	0.2	-0.14	-0.07	0.09
TBWBSF14				1	0.4	-0.12	0.16	0.24
TBWBSF21					1	-0.27	-0.24	0.22
GMWBSF7						1	0.51**	0.35
GMWBSF14							1	0.36
GMWBSF21								1

** = P < 0.01; * = P < 0.05

muscle aged for 14 d but not 21 d.

20 d Heifer LL, TB and GM Correlations. Within the 20-d ZH treatment, WBSF of the LL was not correlated with WBSF of the TB and GM. Correlations within the 20-d ZH treatment for different aging times were moderately (P < 0.05) correlated. The WBSF of GM muscle aged for 7 d was positively correlated (P < 0.01) with heifer GM

Table 6. Correlations among Warner-Bratzler shear force of 20 d heifer longissimus lumborum, triceps brachii and gluteus medius muscles aged for 7, 14 and 21 d

Column1	LLWBSF14	LLWBSF21	TBWBSF7	TBWBSF14	TBWBSF21	GMWBSF7	GMWBSF14	GMWBSF21
LLWBSF7	0.81**	0.87**	0.05	0.19	-0.29	0.16	0.12	0.11
LLWBSF14	1	0.87	0.12	0.06	-0.25	0.18	0.25	0.13
LLWBSF21		1	-0.010	-0.05	-0.26	0.23	0.12	0.22
TBWBSF7			1	0.60**	0.55**	0.09	0.20	0.08
TBWBSF14				1	0.47*	0.02	0.21	0.14
TBWBSF21					1	0.02	0.13	0.11
GMWBSF7						1	0.66**	0.28
GMWBSF14							1	0.35
GMWBSF21								1

30~d~Heifer~LL, TB~and~GM~Correlations. The WBSF of 30 d heifer TB muscle aged for 14 d was positively correlated (P ≤ 0.01) with TB muscle aged only for 7 d. The WBSF of 30 d heifer GM muscle aged for 14 d was positively correlated (P < 0.05) with heifer GM muscle aged for 21 d. Unlike with the C and 20 d treatments, the WBSF of GM muscle aged for 14 d was positively correlated (P ≤ 0.05) with WBSF of LL muscle aged for 21 d was positively correlated (P < 0.05) with WBSF of LL muscle aged for 7 and 14 d. These results indicate that as WBSF of LL muscles aged for different times increase, the WBSF of GM muscles aged for specific times increase as well, but the TB does not.

Table 7. Correlations among Warner-Bratzler shear force of 30 d heifer longissimus lumborum, triceps brachii and gluteus medius muscles aged for 7, 14 and 21 d

Column1	LLWBSF14	LLWBSF21	TBWBSF7	TBWBSF14	TBWBSF21	GMWBSF7	GMWBSF14	GMWBSF21
LLWBSF7	0.82	0.66	-0.04	-0.19	-0.13	0.17	0.42	0.43
LLWBSF14	1	0.75	0.11	0.06	-0.18	0.15	0.51	0.58
LLWBSF21		1	0.10	0.06	-0.02	0.10	0.44	0.24
TBWBSF7			1	0.60	0.27	0.20	0.38	0.004
TBWBSF14				1	0.02	-0.15	0.14	-0.002
TBWBSF21					1	-0.31	-0.10	-0.02
GMWBSF7						1	0.30	0.35
GMWBSF14							1	0.47

40d Heifer LL, TB and GM Correlations. The WBSF of 40 d heifer LL muscle aged for 21 d was negatively correlated (P < 0.05) with WBSF of TB muscle aged for 7 d. I have no explanation for this inverse relationship. None of the GM by aging time WBSF values were correlated (P > 0.05) with LL WBSF values at any aging time. This contradicts the significant correlations for heifers fed ZH for 30 d.

Table 8. Correlations among Warner-Bratzler shear force of 40 d heifer longissimus lumborum, triceps brachii and gluteus medius muscles aged for 7, 14 and 21 d

Column1	LLWBSF14	LLWBSF21	TBWBSF7	TBWBSF14	TBWBSF21	GMWBSF7	GMWBSF14	GMWBSF21
LLWBSF7	0.55	0.55	-0.30	-0.11	0.13	0.13	0.08	0.38
LLWBSF14	1	0.64	-0.42	-0.26	0.07	0.37	0.33	0.15
LLWBSF21		1	-0.46	-0.07	-0.06	0.02	0.14	0.07
TBWBSF7			1	0.26	0.21	-0.14	-0.33	-0.28
TBWBSF14				1	0.23	0.26	0.32	0.40
TBWBSF21					1	0.25	-0.21	-0.03
GMWBSF7						1	0.44	0.59
GMWBSF14							1	0.34

Summary

The WBSF of all muscles tested for steers and heifers increased (P < 0.05) from feeding ZH 0 to 40 d. Adding ZH in the diet for 30 d resulted in all muscles of steers and heifers tested to have WBSF means that were numerically lower (P > 0.05) than 40 d of treatment. Aging of these muscles from steers and heifers that were fed ZH for 30 and 40 d can help to mitigate some of the negative affect of ZH on tenderness; however, the WBSF values were still numerically higher than those of muscles from cattle that were fed ZH for 0 or 20 d. Several studies have found that feeding ZH for 30 or 40 d has benefits on efficiency of growth and carcass composition, but I found that it also negatively impacted tenderness of steer LL and heifer LL, TB and GM muscles. The WBSF means for 30 and 40 d treatment for all muscles were < 5 kg and aging for 21 d brought these means down to < 4.4 kg. Although the means appear to be of acceptable tenderness, there were several steaks from cattle fed ZH for 30 or 40 d that had WBSF values \geq 5 kg, whereas none of the steaks from C or 20 d ZH cattle had values \geq 5 kg. Longissimus lumborum steaks aged for 21 d from heifers treated for 40 d had 6.6% with WBSF values \geq 5 kg. GM steaks aged for 21 d from heifers treated for 40 d had 10.7 %

with WBSF values ≥ 5 kg. Treatment with ZH for 20 d in heifers resulted in WBSF values that were not different (P > 0.05) from C for the LL and GM muscle. Feeding ZH for 20 d to steers resulted in WBSF values for the LL that were higher (P < 0.05) than C, but lower (P < 0.05) than the 40 d treatment. Any treatment of ZH to heifers increased (P < 0.05) WBSF of the TB muscle the same amount; however, the WBSF means for all treatments were not above 4.4 kg.

Treatment of steers and heifers with 20 d ZH and 21 d of aging resulted in steer LL and heifer TB having none of its steaks with WBSF ≥ 5 kg. Differences between WBSF of heifers versus steers could not be statistically analyzed; however, the WBSF means of heifer LL were numerically higher (.5 to .9 kg among treatments) than the steer LL WBSF values. These results agree with those of Choat et al. (2006) and Tatum et al. (2007) who found that steers typically produce more tender meat than heifers. When looking at the effects of ZH on the four muscle x gender combinations, it appears that they are affected differently. The LL muscle of steers and heifers both increased 1.2 kg from 0 to 40 d of treatment; heifer GM muscle increased .6 kg from 0 to 40 d; and heifer TB muscle increased .5 kg from 0 to 40 d of treatment. These results are consistent with those of Strydom and Nel (1999) who reported that the effects of ZH on meat quality are muscle specific.

There were no differences (P > 0.05) in % IMF within steer LL, heifer TB or heifer GM muscles due to ZH treatment.

There were several treatment combinations that showed a significant (P < 0.05) correlation between % IMF and LM area or APYG. Steaks from 20 d steer LL, C heifer LL, 20 d heifer LL, 30 d heifer LL, 20 d heifer TB and 20 d heifer GM, all had negative correlations between LM area and % IMF. This supports the theory that ZH is a repartitioning agent that moves nutrients away from fat and towards muscle synthesis. There were more significant correlations between LM area and % IMF for the LL muscle than any of the other muscles. There is little research that has been done on the effects

of ZH or any β -AA on different types of muscle. The LL muscle in my study may have had more significant correlations between LM area and % IMF because the LL muscle has more intramuscular fat, so it may be able to repartition more of these nutrients to increased muscle protein synthesis. Steaks from 30 d heifer LL, 30 d heifer TB, 40 d heifer TB, 20 d heifer GM and 30 d heifer GM all had positive (P < 0.05) correlations with %IMF and APYG. These results are to be expected if β -AA act by decreasing fat in different depots. The 30 d steer LL, 40 d steer LL, C heifer LL, 20 d heifer LL, 30 d heifer LL, C heifer TB and 30 d heifer GM all had negative correlations between WBSF and % IMF. These results indicate that within a treatment x muscle x aging combination, % IMF may have an impact on tenderness. When % IMF decreases, WBSF increases, likely because muscle causes more shear resistance than fat; when less fat is present, there is more muscle protein.

Implications

Supplementing feedlot diets with Zilmax for 20, 30 or 40 d will increase WBSF of both steer LL and heifer TB muscles, whereas supplementing with Zilmax for 30 or 40 d will increase WBSF of heifer LL muscles. Supplementing diets for 40 d will increase WBSF of heifer GM muscles, even with 21 d of aging.

Previously proven beneficial effects of supplementing feedlot diets with Zilmax on growth and carcass composition must be balanced with the negative effects on tenderness. When Zilmax is fed to capitalize on its growth and composition benefits, only 20 d supplementation is recommended.

Literature Cited

- Avedano-Reyes, L. V. Torres-Rodriguez, F. J. Meraz-Murill, C. Perez-Linares, F. Figueroa-Saavedra, and P.H. Robinson. 2006. Effects of two beta-adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers. J. Anim. Sci, 84:3259- 3265.
- Beerman, D. H. 2004. Beta-agonist. Pages 1026-1030 in Encyclopedia of Meat Sciences. W. K. Jensen, C. Devine, and M. Dikeman, eds. Elsevier, Ltd., Oxford, UK.
- Brooks, C. J. 2008. Zilpaterol hydrochloride effects on red meat yield and quality. Proc 61st Recip. Meat Conf., Gainesville, FL.
- Gruber, S. L., J. D. Tatum, T. E. Engle, M. A. Mitchell, S. B. Laudert, A. L. Scroeder and W. J. Platter. 2007. Effects of ractopamine supplementation on growth performance and carcass characteristics of feedlot steer differing in biological type. J. Anim. Sci. doi:10.2527/jas.2006-634.
- Koohmaraie M. and S. D. Shackelford. 1991. Effect of calcium chloride infusion on the tenderness of lambs fed a beta-adrenergic agonist. J. Anim. Sci. 69:2463-2471.

- Mersmann, H. J. 1998. Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. 76:160-172.
- Miller, M. F., C. R. Kerth, J. W. Wise, J. L. Lansdell, J. E. Stowell, and C. B. Ramsey. 1997. Slaughter plant location, USDA quality grade, external fat thickness, and aging time effects on sensory characteristics of beef loin strip steak. J. Anim. Sci. 75:662-667.
- NCBA (National Cattlemen's Beef Association) 2006. Industry guide for beef aging.

 National Cattlemen's Beef Association. Englewood, CO.
- Plascenia A., N. Torrentera and R. A. Zinn. 1999. Influence of the beta-agonist ZH, on growth performance and carcass characteristics of feedlot steers. J. Anim. Sci. 77(Suppl. 1):114.
- Pringle, T.D., C. R. Calkins, M. Koohmaraie, and S. J. Jones. 1993. Effects over time of feeding a β-agonist to wether lambs on animal performance, muscle growth, endogenous muscle proteinase activities, and meat tenderness. J. Anim. Sci. 71:636-644.
- Schiavetta A. M., M. F Miller, D. K. Lunt, S. K. Davis and S. B. Smith. 1990. Adipose tissue cellularity and muscle growth in young steers fed the β-adrenergic agonist clenbuterol for 50 days and after 78 days of withdrawal. J. Anim. Sci. 68:3614–3623.

- Smith, S. B., D. K. Lunt, and M. Zembayashi. 2000. Intramuscular fat deposition: The physiological process and the potential for its manipulation. Pages 1–11 in Proc. Plains Nutrition Council Spring Conf., Amarillo, TX. Texas A&M Res. Ext. Ctr., Uvalde, TX.
- Strydom, P. E., L. Frylinck, and G. L. Marais. Supplemental vitamin D3 and electrical stimulation to reduce the effect of beta agonists on meat quality. 2007. Pages 273-274 in Proc. 53rd Int. Congr. Meat Sci. Technol., Beijing, China.
- Strydom, P. E., and E. Nel. 1999. The effect of supplementation period of a beta-agonist (zilpaterol), electrical stimulation, and aging period on meat quality characteristics.

 Pages 58-59 in Proc. 45th Int. Congr. Meat Sci. Technol., Yokohama, Japan.
- Vasconcelos J. T., R. J. Rathmann, R. R. Reuter, J. Leibovich, J. P. McMeniman, K. E. Hales, T. L. Covey, M. F. Miller, W. T. Nichols and M. L. Galyean. Effects of duration of zilpaterol hydrochloride feeding and days on the finishing diet on feedlot cattle performance and carcass traits. J. Anim Sci. 1910. doi:10.2527/jas.2008-1032
- Walker, D. K., E. C. Titgemeyer, J. S. Drouillard, E. R. Loe, B. E. Depenbusch, and A. S. Webb. 2006. Effects of ractopamine and protein source on growth performance and carcass characteristics of feedlot heifers. J. Anim. Sci. 84:2795-2800.

Wheeler, T. L. and M. Koohmaraie. 1992. Effects of the β-adrenergic agonist L_{644,969} on muscle protein turnover, endogenous proteinase activities, and meat tenderness in steers. J. Anim. Sci. 70:3035-3043.

Appendix A

Diet composition¹, % of DM

	D	iet
Item	Control	Zilmax ²
Corn, steam flaked	72.9	72.9
Corn DDGS	9.9	9.9
Silage, Corn	10.1	10.1
Tallow	3.0	3.0
Supplement	4.0	4.0
Micro-ingredients ³		
Rumensin, g/ton	33.8	
Tylan, g/ton	9.8	
Zilmax, g/ton		7.56
Vitamin A, IU/lb	1,400	1,400
Vitamin D, IU/lb	140	140
Vitamin E, IU/lb	5	5

¹Fed October 27, 2007 through the end of the trial, including the 3 day withdrawal following Zilmax feeding.

²Fed to Zilmax treatments for 20, 30 or 40 days as stated in the protocol.

³Added using a micro-weigh machine (Micro Beef Technologies, Amarillo, TX)