

EFFECTS OF GROWTH-PROMOTING  
TECHNOLOGIES ON BEHAVIOR, MOBILITY,  
HEALTH PARAMETERS AND HEAT STRESS OF  
FINISHING STEERS

By

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Title of Study: EFFECTS OF GROWTH-PROMOTING TECHNOLOGIES ON BEHAVIOR, MOBILITY, HEALTH PARAMETERS AND HEAT STRESS OF FINISHING STEERS

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**Abstract:** Crossbred steers (n=336; initial BW=379± 8kg) were used in a RCBD to determine the effects of growth-promoting technologies on steers' behavior, mobility, health parameters and heat stress. Treatments consisted of an all-natural treatment (**NAT**), a conventional treatment (implanted with 40mg of estradiol and 200mg of trenbolone acetate (TBA), and fed 33 and 9mg/kg of monensin and tylosin daily, **CONV**) and a CONV treatment plus the addition of zilpaterol hydrochloride (ZH; at 6.8g/ton [90% DM-basis] for the last 20 days on feed with a 3 to 4 d withdrawal; **CONV-Z**). Chute exit scores resulted in a treatment time interaction ( $P= 0.03$ ), with NAT steers having a more aggressive exit score than CONV and CONV-Z steers at d 10Z and d 20Z. There were no effects of treatment on exit velocity, pen temperament, or overall temperament ( $P\geq 0.26$ ). Standing time and lying bouts were not affected by treatment ( $P> 0.45$ ), but CONV-Z steers took more steps/d ( $P= 0.04$ ), resulting in a greater motion index ( $P= 0.05$ ) than NAT steers. While moving to the working facilities, CONV-Z steers moved at the slowest velocity, CONV were intermediary, and NAT the fastest ( $P< 0.05$ ). Step length and mobility scores were not affected by treatment ( $P\geq 0.14$ ). White blood cell counts were greater for CONV and CONV-Z versus NAT steers from d 28 through d 20Z ( $P< 0.05$ ). Liver abscesses, lung scores and heart and liver histological changes were not affected by treatment ( $P\geq 0.10$ ). During summer heat stress, body temperature was not affected ( $P> 0.10$ ), but respiration rate was greatest for CONV-Z steers, intermediate for NAT and lowest for CONV steers ( $P< 0.05$ ). Hair covering scores was lower for CONV and CONV-Z versus NAT cattle from d 84 through d 20Z. The results of this experiment suggest that growth-promoting technologies have little to no overall effects on cattle behavior, mobility and health parameters. Treatment altered the mechanism by which steers exchange heat load to maintain thermo-homeostasis, but all steers experienced a similar magnitude of heat stress. Collectively, growth-promoting technologies did not have a negative effect on finishing steer well-being during this study.

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## CHAPTER I

### INTRODUCTION

In an effort to meet growing protein demands and offset a dwindling U.S. cow herd, the U.S. beef industry has increased the adoption of FDA approved growth-promoting technologies (i.e., growth implants and beta-adrenergic agonists; BAA) to produce more beef. As of 2011, more than 94% of U.S. feedlot cattle were receiving some type of steroidal implant (NAHMS, USDA 2011); and as of August 2013, approximately 60 to 70% were receiving some type of BAA. As a result of improved production efficiency, utilizing these technologies can have multiple benefits: increased beef supply, reduced cost to consumers, reduced usage of natural resources, and reduced greenhouse gas production (Avery and Avery, 2007; Lawrence and Ibarburu, 2006). Improved feedlot performance and carcass characteristics have been well documented with these products. However, there is limited research relative to the effects of these technologies on animal behavior, mobility, health and heat stress in cattle.

Power of Meat (2014) reported that first quarter natural/organic beef sales have increased 2.6% versus one year ago, while total pounds of beef sold have decreased 1.2%. Consumer demand for natural/organic products has rapidly increasing the past 5 years. When surveyed, one of the consumer's top responses for purchasing natural/organic products was "perceived benefits in animal welfare" (Power of Meat, 2013). Implanting cattle with exogenous growth promotants and supplementing BAA will modify nutrient partitioning, increasing growth rate and protein anabolism. This may impact the ability for the immune system to respond to stressors because actions of the anabolic hormone increase protein anabolism and modify metabolism to enhance growth factors in exchange for energy and protein required for immune responses (Richeson et al., 2013). Increases in

body mass in relation to surface area could lead to a greater risk of heat stress. Beta-adrenergic agonists cause arteriole dilation, which has led to increased heart and respiration rates (RR; Bruckmaier and Blum, 1992; Eiler, 2004). How does this all relate to your sentence on consumer responses to purchasing natural/organic products?

The death of finishing feedlot cattle is a rare event, but recent anecdotal reports have generated concern that growth technologies (specifically BAA) may be linked to increases in cattle morbidity and mortality, especially during hot environmental conditions. Extensive research has been conducted examining the effects of growth implants, BAA and other technologies such as ionophores on feedlot performance and carcass characteristics. However, there is limited research that has analyzed the effects of these technologies on animal behavior and welfare in feedlot cattle. To date, the body of literature on growth promoting technologies and livestock behavior and welfare is limited; so the objective of this study was to examine the effects of conventional beef production systems with and without the use of such technologies on behavior, mobility, health and heat stress of finishing steers compared to an all-natural production system.

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## CHAPTER II

### REVIEW OF LITERATURE

#### TECHNOLOGY USE IN THE FEEDLOT INDUSTRY

##### *History of Technologies*

Beef producers in the U.S. have used anabolic implants to improve growth and gain efficiency of cattle since the first diethylstilbestrol (DES) implant was approved in 1956. Growth-promoting implants are broadly categorized on the basis of active ingredients as either estrogenic, androgenic, or a combination implant. Currently, there are two forms of steroidal estrogens that are commonly used as active ingredients: the naturally occurring estrogen ( $E_2$ ) and the modified estrogen, estradiol benzoate (EB). Estradiol benzoate has approximately 71.4% the estrogenic activity of  $E_2$  (Botts, 1997). Though not a steroid, the synthetic  $\beta$ -resorcylic acid lactone, ‘zeranol’ (approved in 1969), possesses estrogenic activity in cattle and is approved for use as an active ingredient in the implant Ralgro<sup>®</sup>. However, zeranol has a lower binding affinity for the estrogen receptor so its estrogenic activity is approximately 15 to 30% of  $E_2$  (Katzenellenbogen et al., 1979). Implants containing zeranol,  $E_2$ , or EB are classified as estrogenic.

Androgenic implants are more commonly used to improve growth and efficiency of heifers and the active ingredients used in those implants could include a naturally occurring testosterone propionate (TP) or a synthetic compound trenbolone acetate (TBA). The androgenic and relative anabolic activity of TBA is 3-5 and 8-10 times greater than TP, respectively (Bouffault and Willemart, 1983). Combination implants include  $E_2$  or EB plus TBA at various doses and were first FDA approved in 1991 (Johnson et al., 2013).

Growth-promoting technologies also include  $\beta$ -adrenergic agonists (BAA) compounds, which are also repartitioning agents that redirect nutrients away from fat deposition in favor of muscle deposition. In the U.S., there are 2  $\beta$ -adrenergic agonists currently approved for use in both steers and heifers fed in confinement for slaughter. Ractopamine hydrochloride (RH), marketed as Optaflexx45<sup>®</sup> (Elanco Animal Health, Greenfield, IN), was approved by the FDA for use in cattle June 13, 2003. Zilpaterol hydrochloride (ZH), marketed as Zilmax<sup>®</sup> (Merck Animal Health, De Soto, KS), was approved by the FDA for use in cattle August 10, 2006. Ractopamine hydrochloride is approved to be supplied at a dietary concentration of 10 to 30 mg/kg of DM at a dose of 70 to 430 mg·hd<sup>-1</sup>·d<sup>-1</sup> for the last 28 to 42 d of the finishing period. Zilpaterol hydrochloride is approved to be supplied at a dietary concentration of 8.33 mg/kg of DM at a dose of 60 to 90 mg·hd<sup>-1</sup>·d<sup>-1</sup> for the last 20 to 40 d of the finishing period with a 3-d withdrawal before slaughter.

The majority of the research involving various BAA indicate a more direct mode of action occurs at specific tissues through  $\beta$ -adrenergic receptors (BAR) rather than indirect actions through circulating hormones (NRC, 1994). Structural differences among various BAA compounds allow for the possibility of different modes of action among BAA, and research investigating the indirect effects of approved BAA is limited, especially with regard to ZH. Feeding BAA results in increased protein deposition, and as a function of increased nitrogen retention, urea nitrogen concentrations would be expected to decrease (Johnson et al., 2013).

Other technologies commonly fed to feedlot cattle include monensin and tylosin. Monensin (most commonly Rumensin<sup>®</sup>, Elanco Animal Health) is an ionophore that was approved for use in the mid 1970's and is commonly used to increase feed efficiency. Tylosin (Tylan<sup>®</sup>, Elanco Animal Health) was approved in the 1960's and is commonly utilized as a feed grade antibiotic to help prevent liver abscesses and improve performance and efficiency.

### ***Current Utilization of Technologies***

The utilization of technologies in animal agriculture has been widespread and the vast majority of those adopting technologies are feedlot producers. According to the USDA National Animal Health Monitoring System (NAHMS) Feedlot study in 2011, 90.1% of all feedlot cattle were fed Rumensin<sup>®</sup>, 71.2% received Tylan<sup>®</sup>, 94.7% of cattle greater than 318 kg received at least one growth implant, and 47.5% were fed a BAA. It has been estimated that greater than 60% of feedlot cattle were receiving a BAA prior to concerns of animal welfare reported in August 2013. Over the years, the adoption of new technologies has been very rapid, but the utilization of these technologies continues to receive more and more push back from the public. The public is concerned with how these technologies may negatively affect the wholesomeness of beef, the environment and the animal's welfare. These areas have been researched minimally and there is a lack of industry-driven education about these technologies and their benefits for consumers.

## **ANIMAL WELFARE AND HEAT STRESS**

### ***History of Animal Welfare***

Animal welfare has been defined as the physical and psychological well-being of animals given their environment, management, nutrition, health, and interaction with humans and other animals. To review the first records of debate concerning the proper treatment of animals, you must look back to Greece beginning in the sixth century BC. Many philosophers like Aristotle and Pythagoras kept records of animal behaviors and were some of the first individuals involved in the debates of animal welfare. At this time, the debate was more centered around if an animal deserved ethical treatment or not. The norm was that animals were not rational thinkers and had no knowledge of the situations to which they were exposed. Therefore it did not matter how the animal was treated. Animals were simply thought of as objects. Many people utilized these thoughts to justify cruel and harsh treatment. Early radical voices claimed that animals were rational thinkers that were made from the same elements, breathed the same air, and were animated by the same reincarnated souls as humans (Fraser, 2008).

Porphry, made arguments that animals were rational thinkers and noted that they lived orderly, rational lives. Porphry added that killing animals for pleasure was unjust, and was the first to make claims that have become the center of animal welfare discussions today. He stated, “Animals deserve moral consideration because they, like us, have the capacity to feel distress, to be afraid, to be hurt, and therefore to be injured” (Fraser, 2008).

Looking ahead to three or four centuries prior to the mid 1900’s, England was a very cruel place in the 16<sup>th</sup>, 17<sup>th</sup>, and 18<sup>th</sup> centuries. Human punishments consisted of hangings, cutting off limbs, and other brutal measures. At that time in history in Britain, cruelty to animals was simply an aspect of daily life. In the mid 1700’s, a British artist William Hogarth produced four pieces of art work that displayed the current acts of animal cruelty and they were widely dispersed. These depictions and other progressive movements gradually shifted the attitudes people had toward animals in the 1700’s as part of a general awakening of feeling pity, kindness, and moral sense. In the late 1700’s, more discussion and concerns related to the moral and ethical treatment of animals circulated. The first attempt to make legislative reform was in 1800 with an attempt to ban “bull-baiting”. Legislative progress was made in the 1800’s in the area of “Ill-treatment” of animals (cruelty topics), but mainly focused on horses, ponies and dogs.

Focus on animal welfare concerns became less of a priority during the early 1900’s as two World Wars and the Great Depression took place. During this era, most of society was concerned with survival and having enough food to feed their family. At this point, survival concerns trumped any thoughts of how the animals were being raised. In the mid to late 1900’s, a publication by Ruth Harrison (*Animal Machines*) and Peter Singer (*Animal Liberation*) focused on the inhumane and unethical treatment they believed confined farm animals experienced in the way they were housed, treated and harvested. These books were very influential and sparked moral concerns and debates that focused on the “quality of life” for animals being raised for food or used in experimentation. These discussions were different than previous debates in Greece and England when the concerns were more about the “justice” of treating animals’ right and improving the “moral tone of society”,

respectively. The other major difference that occurred in the late 1900's was that animal welfare concerns were starting to be addressed with scientific investigation.

Today's thoughts and concerns are similar, but more intense and extreme than those of the 1960 era. Society is mostly concerned with the justice for the animal, how they are treated and the quality of life they are provided. Since the 1960's, society has continued to use more and more science to study and understand animal welfare and what should or should not be morally acceptable. Society has made considerable strides in this field, yet there is still a lot to learn. Currently the U.S. society is stricter than it has ever been in terms of their thoughts regarding moral treatment of animals. Today, animal activities are subject to verification and rational defense in order to meet the moral acceptance of that study or activity. The number of animal activist groups that believe it is immoral to utilize animals for any human use (food, research, labor, companionship, education, etc.) is growing and these groups are gaining financial and emotional support today more than ever before. Over the last decade, these groups have been imposing their beliefs on animal industries in the U.S. and changing the way those operations function. Europe, on the other hand, is stricter and more tightly regulated than the U.S. by agencies that provide "moral" guidelines for production and research.

### ***Factors Affecting Heat Stress***

Heat stress can ultimately be defined as an event when total heat gain exceeds an animal's heat loss capabilities, causing increased body temperature, disrupted behaviors and impaired physiological function (Hahn and Becker, 1984). By definition, the most ideal method of measuring heat stress is to record core body temperature, but monitoring body temperature is not feasible or realistic for large numbers of cattle in a commercial production setting. Other indicator traits used to quantify heat stress include assigning panting scores (PS), quantifying respiration rates (RR), or both (Gaughan et al., 2000; Silanikove, 2000). Many different indices have been developed since 1959 to use environmental conditions as indicators of thermal stress in livestock. These indices would

include: temperature-humidity index (THI; Thom, 1959), Livestock Weather Safety Index (LWSI; Livestock Conservation Incorporated, 1970), heat load index (HLI; Gauhan et al., 2007), and most recently, comprehensive climate index (CCI; Mader et al., 2010). These indices started with the THI that adjusted ambient temperature based on humidity and have progressed to the CCI that adjust ambient temperature based on humidity, wind speed and solar radiation.

**Environment.** Ambient temperature is the main environmental factor affecting heat stress in cattle. Based on temperature alone, there would be non-evaporative heat transfer which is proportional to the temperature gradient within the animal and between the animal and the environment (Morrison, 1983). Dissipating heat via evaporation is directly related to the relative humidity (RH) level. As RH, increases it becomes more difficult to dissipate heat through water vapor (from sweating or respiration; Mader, 2003). Solar radiation is also a contributing factor as it can drastically increase the animal's skin temperature, restricting the animal's ability to transfer heat from the core body out through skin the surface. Wind speed is a factor than affects heat dissipation at the animal's surface. As air travels over the animal's surface, it moves hotter air away from the animal and pulls heat out of the skin. Moisture (i.e. rain) would serve as a great coolant. Due to its high value of specific heat, water is a great storage molecule for heat. Currently, none of the environmental indices account for moisture as it relates to an animal's surface becoming wet by some type of precipitation (Mader et al., 2010).

**Breed Type and Hair Coat Characteristics.** *Bos indicus* cattle are considered more heat tolerant than the *Bos taurus* breeds. The majority of these differences stem from genetic variations that result in the expression of phenotypes that are able to dissipate more heat. Approximately 85% of an animal's heat load must be exchanged through the skin and 15% via the respiratory tract (Finch, 1986). Finch (1985) explained that *Bos indicus* cattle have an increased ability to dissipated heat through the skin, resulting in reduced heat storage. This fact can be attributed to many factors including less subcutaneous tissue, thinner hide, increased skin surface area, increased blood flow to the surface, and a smoother, shorter, more reflective hair coat. Hair coat characteristics play a

significant role in heat exchange. Rougher hair coats act as insulation to trap heat within the body. They can also accumulate water vapor (from sweat and humidity) at the skin's surface, limiting the ability for animal to cool through evaporation. Rough and dark colored hair coats also increase the absorption of solar radiation, which rapidly increase the skin temperature (Finch, 1986). Indicators of heat stress (i.e. RR and body temperature) are typically greater in dark-hided cattle (Arp et al., 1983; Mader et al., 2006). Rectal temperatures averaged 0.3°C greater in black *Bos taurus* versus white *Bos taurus* cattle (Finch et al., 1984). Mader et al. (2002) and Davis et al. (2003) reported a 0.2 to 0.6°C increase in body temperature of dark- versus light-colored cattle. These differences have been attributed to the greater heat flux present at the skin of darker-haired animals. Busby and Loy (1996) concluded that greater than 75% of feedlot deaths caused by heat stress were dark-coated cattle.

**Diet and Feed Intake.** Dietary manipulation may be one of the least expensive and most beneficial strategies for helping cattle cope with environmental stress (Hahn, 1995; Mader et al., 1999). Restricting feed intake causes a decrease in body temperature (0.5°C; Mader et al., 2002). Purwanto et al. (1990) suggested that DMI is partially responsible for total heat production and reduced intake should decrease maintenance heat production. Organ size and metabolic rate are also likely contributors to variation in body temperature. Various planes of nutrition can alter maintenance requirements which are commonly linked to changes in metabolism or in the size of metabolically active organs (Koong et al., 1985; Burrin et al., 1990; Freetly et al., 1995). In addition, heat production during fasting has been shown to decrease if cattle were previously consuming a lower DMI (Graham and Searle, 1972; Graham et al., 1974). Dye-Rose et al. (2009) concluded that diet type also altered heat load, as measured by ruminal temperature. That experiment demonstrated that cattle fed receiving/growing rations (16 to 40% alfalfa) had a lower (0.2°C) average rumen temperature than cattle fed a finishing ration (6% alfalfa). In this case, heat of fermentation and maintenance heat production were likely contributors to the change in body temperature. Although altering feeding time and intake amounts have consistently reduced heat stress (body temperature,

respiration rates, etc.) overall performance and feed efficiency have not been altered by these strategies (Mader et al., 2004).

**Management.** Providing shade, wetting the cattle and wetting the soil surface have all been investigated as methods to mitigate heat stress. All three methods are successful at reducing heat load of cattle, but a reduction in heat load has not always translated to an improvement in performance or efficiency. Shade is likely the most reliable method of reducing heat stress (Bond et al., 1967; Valtorta et al., 1997; Gaughan et al., 1998; Brown-Brandl et al., 2005) and decreasing heat-related mortalities across a variety of environmental conditions (Busby and Loy, 1996; Entwistle et al., 2000). Performance results, however, are reported to vary. Mitloehner et al. (2002) and Gaughan et al. (2010) reported that shade significantly increased DMI (3%) and ADG (6 to 9%) during heat stress periods, while others have reported no performance differences between shade and no shade (Clarke and Kelly, 1996; Mader et al., 1997). Misting cattle was relatively ineffective in regard to relieving heat stress or improving performance (Mitloehner et al., 2001). On the other hand, sprinkling cattle is a viable option for mitigating heat stress and promoting DMI (12%) and ADG (20%) (Morrison et al., 1973; Morrison, 1983). Mitloehner et al. (2001) concluded that sprinkling is more effective than misting at ameliorating the effects of heat stress. This is due to fine water droplets clinging to the outer hair and never reaching the skin's surface. As a result, the mist layer acts as an insulator that traps hot air next to the surface and reduces heat exchange to the environment. Completely wetting the skin surface alleviates heat stress due to the latent heat of vaporization associated with the change of water from liquid to a gaseous state at the surface (Mader and Davis, 2004). Wetting the pen surface has been shown to cool the surface by 15°C. This reduction in temperature can create a temperature gradient that favors the transfer of heat from the animal's core to the skin surface. In addition, dry soil surfaces have a thermal conductivity of 0.25 W/m<sup>2</sup>, but the thermal conductivity is increased 5-fold when the surface is wet allowing for more heat exchange at the surface (Campbell et al., 1994).

## IONOPHORES AND ANTIBIOTICS

### *Mechanism of Action*

Feed-grade ionophores and antibiotics are two technologies that are commonly used in feedlot cattle. Rumensin<sup>®</sup> is the trade name for monensin, which is the primary ionophore utilized in feedlot cattle for increasing feed efficiency and performance. Monensin is a carboxylic polyether ionophore (Haney and Hoehn, 1967) that transports metal ions and protons across the cellular membrane (Thomas, 2006) of ruminal microorganisms. First, monensin will attach to the cell membrane of Gram-positive ruminal microorganisms, causing a loss in cellular potassium and an influx of H<sup>+</sup> ions which reduces cellular pH (Russell, 1997). Sodium will flow into the cell and the microorganism attempts to pump H<sup>+</sup> ions out. The response to pump protons out depletes cellular adenosine triphosphate (ATP) and the lack of ATP limits cell growth and reproduction, which ultimately leads to cell death (Thomas, 2006). Differences in cell membrane characteristics, cause gram-positive bacteria to be more sensitive to monensin than gram-negative.

Ultimately, ruminant metabolism is impacted by increasing the efficiency of energy metabolism, improving nitrogen utilization, and reducing bloat and lactic acidosis (Schelling, 1984). The profile of volatile fatty acids is altered because gram-negative microorganisms produce more propionic acid and consequently reduces the molar percentages of acetic and butyric acids (Prange et al., 1978). Propionate is either used directly by the animal for energy or increases the hepatic gluconeogenic flux. Monensin collectively allows the animal to produce and utilize more energy from feedstuffs. Additionally, when propionate is increased in relation to butyrate and acetate, hydrogen will be reduced thus decreasing methane emissions (Ellis et al., 2012). Another benefit of monensin is its inhibitory effects of lactic acid producing bacteria, such as *S. bovis* and *Lactobacillus* (Cheng et al., 1998), the main drivers of feedlot bloat and lactic acidosis.

Tylosin, the main feed grade antibiotic fed to feedlot cattle, is used to prevent *Fusobacterium necrophorum* and *Actinomyces pyogenes* bacteria growth. These bacteria are targeted due to their correlation with liver abscesses in cattle (Nagaraja and Chengappa, 1998). Tylosin is a macrolide

antibiotic that works to mainly inhibit gram-positive bacteria, however, *F. necrophorum*, a gram-negative bacteria is also susceptible to tylosin. Tylosin crosses the cell membrane and prevents protein synthesis by binding to the L27 protein of the 50S subunit of the bacterial ribosome. This action inhibits the translocation of tRNA from the peptidyl site. Nagaraja and Chengappa (1998) discussed that tylosin primarily reduces the growth of these bacteria in the rumen, but can be effective in hepatic tissue as well. Ultimately, the results are reduced liver abscesses, increased growth and improved efficiency.

### ***Effects on Animal Health***

Many scientists have hypothesized that monensin could improve the health of cattle. Although some studies reported contradictory results, a Meta-Analysis in lactating dairy cattle by Duffield et al. (2008) concluded that monensin decreased the risk of ketosis, displaced abomasums and mastitis. Monensin supplementation had no effect on milk fever, lameness, dystocia, retained placenta or metritis. Based on the action mechanisms of monensin, it has been suggested that improved energy and protein metabolism, and a reduced risk of ruminal acidosis should result in measurable health benefits for cattle, particularly during the transition and finishing phase when the risk of metabolic disease is greatest.

Tylosin can be fed to swine in all stages of production to increase performance and efficiency and control swine dysentery and porcine proliferative enteropathies. Tylosin is fed to beef cattle to reduce the incidence of liver abscesses. In chickens it can aid in the control of chronic respiratory diseases and improve feed efficiency. Coopriider et al. (2011) reported no differences in abscessed livers between natural (13%; received no tylosin) and conventional (11%; supplemented with tylosin) treatments. Maxwell et al. (2014a) described a similar percentage (11%) of abscessed livers in the conventional cattle, but natural fed steers had nearly 40% condemned livers when tylosin was not supplemented. Using tylosin phosphate in feedlot rations has been reported to decrease the incidence of liver abscesses by 40 to 70% (Nagaraja and Chengappa, 1998). In feedlot cattle, tylosin is

commonly fed as a method of reducing liver abscesses, but it can also be utilized as a treatment for bovine respiratory complex, foot rot and calf diphtheria. The dosage for cattle is approximately  $95 \text{ mg} \cdot \text{hd}^{-1} \cdot \text{d}^{-1}$  to reduce liver abscesses, but 8000 mg is the recommended dosage of injectable tylosin to treat bovine respiratory complex in a 454 kg steer.

## **GROWTH-PROMOTING IMPLANTS**

### ***Mechanism of Action***

The nature of hormone release from a growth-promoting implant would cause a spike in blood  $E_2$  and TBA concentrations within 1 to 3 d after implanting, after which  $E_2$  and TBA concentrations follow a depletion curve that aligns with first-order kinetics (Brandt, 1997). However, when  $E_2$  is combined with TBA in a compressed pellet, plasma  $E_2$  concentrations display a similar pattern, but plasma  $E_2$  is elevated (over control) for a greater period, and the decrease in  $E_2$  is not as rapid (Johnson et al., 1996). Therefore, the increased  $E_2$  concentrations later in the feeding period are likely a result of the physical properties of the substances ( $E_2$  combined TBA) when mixed. Concentrations of plasma trenbolone are not affected by the combination of TBA and  $E_2$  in a single implant.

The lipophilic properties of steroids allow for simple diffusion through the plasma membrane and into the cytoplasm of cells where they bind with steroidal receptors located in either the cytosol or nucleus. Both estrogen receptors (ER) and androgen receptors (ANR), when bound to the ligand, can bind to specific response elements on the DNA strand and regulate the transcription of numerous genes. Bovine satellite cell proliferation is stimulated by  $E_2$  and TBA, and these steroids work directly through their receptors. Estrogens also have been shown to work non-genomically (indirectly) through second-messenger signaling mediated by G-protein coupled receptors on the plasma membrane, as well as other messenger pathways.

Androgen receptors (ANR) are present in numerous tissues including bovine skeletal muscle. Androgens bind to the ANR and are able to directly influence cellular functions through both

genomic and non-genomic mechanisms. Treatment of bovine satellite cells with TBA resulted in increased expression of IGF-I and ANR mRNA (Kamanga-Sollo et al., 2004). As with estrogens, anabolic responses through androgenic binding of the ANR are complex and still not completely understood, but ANR regulation of IGF-I expression may be a contributing factor to anabolic responses.

Steroid hormones typically pass through the plasma membrane, and the classical responses of steroids are to work through cytosolic and nuclear receptors as previously described. However, recent research has suggested that estrogens may also work non-genomically through receptors on the plasma membrane. The non-genomic signaling includes increases in cellular 2<sup>nd</sup> messengers such as Ca<sup>2+</sup>, cAMP, nitric oxide, activation of receptor tyrosine kinases and other kinase activity (Revankar et al., 2005). The G-protein coupled receptor GPR30 was found to bind estrogen and binding resulted in Ca<sup>2+</sup> mobilization and kinase activity. Kamanga-Sollo et al. (2008) demonstrated that by incubating bovine satellite cells with E<sub>2</sub> bound to BSA (bovine serum albumin which prevents diffusion through the plasma membrane), cell proliferation was not affected by E<sub>2</sub>-BSA, but IGF-I mRNA expression was increased. These data suggest that E<sub>2</sub>-stimulated proliferation of cells and E<sub>2</sub>-stimulated increased expression of IGF-I may occur through different pathways (Kamanga-Sollo et al., 2008). Part of the anabolic actions of estrogens associated with anabolic implants could be mediated through the membrane bound GPR30 receptor.

The mechanism of action by which estrogens, androgens, and the combination cause increased growth in ruminants has been researched extensively, but contains some unknowns. Growth hormone (GH) and the somatotrophic axis have long been implicated as a major mechanism by which estrogens cause anabolic responses in cattle. Stuempler and Burroughs (1959) demonstrated that oral administration of diethylstilbestrol (DES) to steers resulted in increased anterior pituitary GH synthesis and caused heavier anterior pituitaries. In contrast, GH concentrations of steers were not affected by implants containing TBA alone or mixed with E<sub>2</sub> (Heitzman et al., 1977). Galbraith and Watson, (1978) suggested that TBA may act directly on the muscle or liver and

that the mechanism of action for TBA was not mediated through GH. Hayden et al. (1992) validated that plasma GH concentrations were increased in steers implanted with E<sub>2</sub>, whereas, implanting with TBA alone or TBA + E<sub>2</sub> (separate implants) did not affect GH concentrations. The results from both Heitzman et al. (1977) and Hayden et al. (1992) demonstrate that the stimulation of GH caused by E<sub>2</sub> seems to be blocked by TBA.

Growth hormone stimulates release of IGF-I and causes numerous metabolic responses mediated through IGF-I. Therefore, increased IGF-I would be expected in steers implanted with E<sub>2</sub>. Breier et al. (1988) implanted steers (25.7 mg E<sub>2</sub>) and concluded that plasma GH concentrations were increased by E<sub>2</sub>, independent of the plane of nutrition. Implanting with TBA or TBA and E<sub>2</sub> (separate implants) also resulted in increased IGF-I concentrations, independent of GH concentrations (Lee et al., 1990). Johnson et al. (1996) demonstrated that implanting with E<sub>2</sub> and TBA (combined in the same implant) increased IGF-I and IGF-I binding protein-3. These results of increased GH and IGF-1 will directly affect muscle cells to cause an increase in the recruitment of satellite cells to muscle fibers and increase the amount of protein synthesis.

Sustained hypertrophy of muscle fibers requires that the ratio of protein to DNA remains constant. Any increases in protein synthesis must be followed by increases in DNA, and because postnatal skeletal muscle fibers and nuclei within the fibers do not divide, DNA for protein synthesis must be provided from other sources. Allen et al. (1979) determined that satellite cells are responsible for providing DNA to sustain muscle cell growth. Bovine satellite cells cultured from steers implanted with TBA + E<sub>2</sub> (Revalor-S) had increased proliferation and a shorter lag phase compared with cells from non-implanted steers (Johnson et al. 1996). The effects of steroid hormones on the proliferation of bovine satellite cells may be mediated through other growth factors or directly, as both E<sub>2</sub> and TBA have been shown to increase mRNA expression of IGF-I (Frey et al., 1995; Johnson et al., 1998; Kamanga-Sollo et al., 2004). Estrogens seem to work both indirectly through GH and IGF-I, as well as working directly through receptors on the skeletal muscle. Androgens may have a more direct mechanism of action through their individual receptors.

### ***Effects on Temperament, Behavior and Mobility***

***Temperament.*** Temperament of cattle has been defined as the behavioral responses of cattle when exposed to human handling (Fordyce et al., 1988) or a stressful event (Café et al., 2011). Excitable temperaments have resulted in reduced performance, efficiency, and meat quality (Fordyce et al., 1998; Nkrumah et al., 2007). Extreme or reactive responses could also be detrimental to cattle welfare and the safety of human handlers. From an emotional or feeling-based perspective, good welfare is the maximization of positive emotions and reduction of negative ones (Duncan, 1996).

Angus and Limousin cattle fed for 208 d and implanted with 36 mg of zeranol every 70 d. This implant strategy had no effect on the time for cattle to enter the chute compared to non-implanted counterparts (Vanderwert et al., 1985). Baker and Gonyou (1986) concluded that implanting (36 or 72 mg of zeranol) reduced the time it took cattle to enter the chute pre-castration (0.76 seconds), but post-castration there were no differences between implanted or non-implanted steers. Vanderwert et al. (1985) reported that implantation had no effect on scale temperament score. Baker and Gonyou (1986) agreed with this conclusion post-castration, but pre-castration increasing dosages of zeranol caused a linear increase in chute temperament. Implantation with zeranol versus estradiol-benzoate caused no differences in mean chute temperament scores (Stricklin et al., 1979). Time for cattle to exit the chute was recorded by Vanderwert et al. (1985) and Baker and Gonyou (1986). Neither study detected a treatment effect of implantation on the total time for cattle to exit the chute. Vanderwert et al. (1985) and Baker and Gonyou (1986) analyzed flight distance (closest distance obtained before the animal reacted in any manner) for cattle to assess pen temperament. Both of these studies concluded that zeranol implantation had no effect on flight distance (approximately 1.4 and 1 m, respectively). However, Hawkins et al. (2005) reported positive effects on docility (decreased fighting and aggression toward pen mates) when steers and bulls were implanted with 36 mg of zeranol.

***Behavior.*** Very limited to no data has been published in regard to feedlot cattle behavior (standing time and lying bouts) and activity (steps taken) in their home pen as a result of technology use. The majority of studies that have investigated the effects of implantation were conducted prior to FDA approval of combination implants, so it is important to keep in mind the variations in mechanism of action (previously discussed). Zeranol implants would be considered very mild implants for finishing cattle relative to combination growth implants.

Previous research has utilized scan sampling and video recording to observe pen activity. In a study by Hawkins et al. (2005), young calves were subject to implantation with or without 36 mg of zeranol. Once at the feedlot, these cattle were observed for pen activity (time spent standing or resting) and previous implant status did not affect pen activity (Hawkins et al., 2005). Unruh et al. (1986) utilized an activity score (a composite of activity, restlessness and movement) to quantify the behavioral effects of implanting cattle every 84 d from birth to slaughter with 36 mg of zeranol. Repeated implantation with zeranol did not affect overall activity. Stricklin et al. (1979) reported that implantation with zeranol versus EB and progesterone had no effect on time spent lying or standing. No differences were detected in the number of walking observations (observers counted walking events) during the sampling hours of 0900 to 1500 h in cattle implanted with zeranol or EB (Stricklin et al., 1979).

***Mobility.*** The effect of growth implants on total time of movement, uric acid, step length and mobility scores in beef steers has not been previously published.

### ***Effects on Heat Stress***

Mader and Kreikemeier (2006) concluded that growth promoting treatments (including multiple implant protocols) did not affect mean tympanic temperature of heifers during the summer or winter. Non-implanted heifers and heifers administered a combination implant had an average temperature of 39.0°C. Based on a temperature and humidity index, the heifers in Mader et al. (2014) were experiencing heat stress during most of the summer sampling period.

Mader et al. (2008) reported that growth promoting implants did not have an effect on PS in cattle during hot environmental conditions. Gaughan et al. (2005) reported that growth implants had no effect on steer or heifer RR, with an average of 114 breaths·m<sup>-1</sup> exhibited by cattle. Hair growth and shedding are tightly controlled and regulated by complex mechanisms under hormonal control, but knowledge is lacking in regard to the mode of action and specific hormonal compounds responsible for hair growth and shedding. Preliminary results of a previous experiment suggest that growth-promoting implants (estrogenic and androgenic compounds) promote the shedding of winter hair coats (unpublished data). The inability of natural fed steers to fully shed their winter hair coat can have a significant impact on physiological heat dissipation. Longer, rougher hair coats provide more insulation, resulting in less heat loss from the skin to the environment. These type of hair coats are also less resistant to heat transfer from the skin by solar radiation than smooth, shiny summer coats that reflect more radiation at or near the surface (Finch et al., 1986).

### ***Effects on Animal Health***

Utilizing 1600 steers and a combination implant, Munson et al. (2012) reported no differences in morbidity (26.6%) or mortality (8.5%) when high risk calves were implanted on arrival or delayed 45 d before implantation. Utilizing seven different sequences of growth promoting implants (one control and six treatments), Smith et al. (1999) concluded that the frequency of medical treatment and death loss (2.5%) did not differ across implantation treatments for Holstein calves. Increasing the concentration of zeranol (0, 12, 24 and 36 mg) when implanting sheep caused a linear increase in mortality (0, 6, 11 and 14, respectively; Eckerman et al., 2013) in one study, but no differences in another (Salisbury et al., 2007).

Implantation with 36 mg of zeranol did not alter hematocrit percentages in feeder calves (Phillips et al., 1986). Smith et al. (1999) and Richeson et al. (2013) reported that various implant strategies had no effect on red blood cells, hemoglobin or hematocrits of Holstein calves or stocker beef calves, respectively. Implanting stocker calves with 200 mg of progesterone and 20 mg of EB

had no effect on total or differentiated white blood cell counts (Richeson et al., 2013). Utilizing that same implant in Holstein steers increased the total white blood cell counts versus non-implanted calves, but did not affect the distribution profile of white blood cells (Smith et al., 1999). Recently, Gifford et al. (2014) concluded that utilizing combination (TBA/E<sub>2</sub>) growth promoting implants reduces serum cortisol production. Yeager et al. (2011) proposed a biphasic mechanism of cortisol effect on immune function where varying concentrations of cortisol can be either pro- or anti-inflammatory. High levels of glucocorticoids can suppress the inflammatory response, while moderate levels can increase receptors for pro-inflammatory cytokines, extend neutrophil lifespan, and activate macrophages. Further research is needed to investigate the use of these technologies on the hematology and cell functions of finishing cattle.

In regard to implantation, Angus feeder calves administered 36 mg of zeranol did not affect serum potassium concentration (Phillips et al., 1985). Enright et al. (1993) injected finishing beef heifers daily with growth hormone-releasing factor and/or thyrotropin-releasing hormone in an attempt to achieve a similar response as when utilizing a growth promoting implant. Growth hormones had no effect on glucose concentrations in that experiment. In feeder calves (Phillips et al., 1985) and finishing lambs (Wiggins et al., 1976; Wilson et al., 1972), zeranol implantation did not affect glucose concentrations either. All three of the previously mentioned studies concluded that treatment caused none to minimal (0-5.6%) improvements in performance, compared to a combination implant in which ADG should be improved by 25% versus non-implanted counterparts. These differences could be contributed to the potency of the implant and the variation in mode of action, as previously described. In response to implantation, scientists have reported mixed results depending on the implant protocol, but aggressive combination implants have typically reduced circulating urea nitrogen in finishing heifers and bulls (Bryant et al., 2010; Mader and Kreikemeier, 2006; Istasse et al., 1988). The fact that implants decrease circulating urea nitrogen concentrations can be explained by the mode of action of these products, which promote muscle protein synthesis and utilization of nitrogen (Johnson and Chung, 2007)

With respect to lung and heart health, Munson et al. (2012) reported that 20% of “high risk” calves had greater than 5% pleural adhesion at harvest, irrespective of implantation time. Klindt et al. (1992; 1995) concluded that administration of increasing concentrations of porcine somatotropin linearly increased heart weights in finishing pigs. Alternatively, implanting sheep with TBA/EB did not alter heart weights in lambs fed concentrate or forage diets, but these lambs were only implanted 32 d prior to harvest which may have limited the expected implant response (McClure et al., 2000).

## **$\beta$ -ADRENERGIC AGONIST**

### ***Mechanism of Action***

Hormone-stimulated cellular responses first require the presence of a receptor is available to bind to the specific ligand. Both natural and synthetic catecholamines bind to adrenergic receptors (AR), and these receptors are found on virtually every cell type (Mersmann, 1998). The AR can be separated into 2 types including  $\alpha$ -AR and  $\beta$ -AR (NRC, 1994). The  $\beta$ -AR are expressed by almost every tissue cell type, including skeletal muscle, and are naturally responsive to norepinephrine and epinephrine (Mersmann, 1998). The  $\beta$ -AR are further categorized into  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  subtypes based on their affinity for norepinephrine. These receptors are G-coupled protein receptors that intersect the cell membrane seven times. Binding of a ligand to the  $\beta$ -AR activates the G-proteins and the  $\alpha$ -subunit on the  $G_s$  protein dissociates from the  $G_{\beta\gamma}$  subunit (mediated through GTP). The dissociated  $\alpha$ -subunit of the  $G_s$  protein activates the enzyme adenylate cyclase. Adenylate cyclase catalyzes the reaction of ATP conversion to cAMP. Then cAMP binds to the subunit of PKA, allowing PKA to phosphorylate many different intracellular proteins, resulting in specific cellular responses (Mersmann, 1998).

The actions of both ZH and RH are believed to be mediated primarily through the  $\beta_1$  and  $\beta_2$ -AR, but RH is thought to have a greater affinity for  $\beta_1$ -AR and ZH is thought to have a greater affinity for  $\beta_2$ -AR. Variations among species in response to BAA exist, and these differences are thought to result from growth propensity differences and variation in  $\beta$ -AR numbers among species. In general,

response to BAA among meat-producing species is thought to be cattle  $\approx$  sheep > pigs > chickens (Mersmann, 1998). Other theories explaining differences among species include specificity among BAA for certain species based on receptor concentrations. Cattle tend to express more  $\beta_2$ -AR, and this may explain why performance responses in cattle typically are greater for ZH than RH.

Beta-agonists have been shown to directly affect skeletal muscle and increase muscle hypertrophy. This response may be attributed to increased protein synthesis, decreased protein degradation, or both (Mersmann, 1998). Unlike implants, however, DNA accretion is not occurring at a rate equal to protein synthesis (Mills, 2002). Therefore, muscle hypertrophy is not a permanent effect, and supplementation of BAA is recommended during the finishing phase before slaughter. The exact mechanism of action through which  $\beta$ -agonist increases muscle growth is yet to be completely determined and probably differs among the various  $\beta$ -agonist compounds and receptors.

### ***Effects on Temperament, Behavior and Mobility***

***Temperament.*** Baszczak et al. (2006) reported no difference in entry force score (a measurement of assistance needed to load into the chute) when steers were fed with and without RH during the final 28 days on feed. Baszczak et al. (2006) did conclude that the steers receiving RH entered the chute at a quicker speed than non-supplemented steers. Feeding BAA to pigs has yielded inconsistent results in the time and amount of assistance pigs require to load into a weighing scale. Marchant-Forde et al (2003) concluded that RH fed pigs required 83% more time and 52% more pats, slaps, and pushes from handlers to enter the weighing crate. Opposing results were concluded by Marchant-Forde et al. in 2008 when supplementing R-salbutamol to finishing pigs. That study resulted in no effect of treatment on the physical handler interactions needed to get the pigs onto a weigh scale. These differences may be related to the RH being classified as a  $\beta_1$  BAA and salbutamol is classified as a  $\beta_2$  BAA. Porcine are known to possess higher concentrations of  $\beta_1$  receptors, which could lead to a different magnitude of results.

No differences were detected by Baszczak et al. (2006) in chute temperament score between steers supplemented with and without RH, while experiencing light pressure in the chute. Alternatively, Burson et al. (2014) concluded that cattle receiving ZH exhibited elevated chute temperament scores on d 20Z. In that study a scoring scale of 1 to 5 was utilized and the cattle were also not restrained in the chute's head catch. Exit score was unaltered by BAA supplementation as reported by Baszczak et al. (2006). Burson et al. (2014) did not measure exit score, but the investigators did conclude a treatment  $\times$  time interaction for exit velocity, with ZH supplemented cattle exiting  $0.45 \text{ m}\cdot\text{s}^{-1}$  faster on d 5 of the treatment period, but no differences at d 0, 10, or 20. This variation in data may also be contributed to initial and overall difference in cattle temperament. Burson et al. (2014) reported mean exit velocities from  $2.9$  to  $3.6 \text{ m}\cdot\text{s}^{-1}$ , while the other studies have reported  $1.8$  to  $2.0 \text{ m}\cdot\text{s}^{-1}$  during the ZH supplementation period. The study performed by Burson et al. (2014) was only a 23 d finishing period in which the cattle were handled minimally prior to the start on ZH supplementation.

**Behavior.** In the presence of humans, Marchant-Forde et al. (2008) concluded that BAA had no effect on behavioral responses of pigs. In that study, all pigs were willing to spend similar amounts of time close and/or touching the human observer. Marchant-Forde et al. (2003), Marchant-Forde et al. (2008), and Athayde et al. (2013) all concluded that BAA supplementation had no effect on the time pigs spent standing. However, when supplementing a higher dose of  $20 \text{ mg/kg}$  of RH, Schaefer et al. (1992) determined that finishing pigs tended to spend 34% less walking/investigating than non-supplemented pigs. The lower doses of  $10$  and  $15 \text{ mg/kg}$  did not affect walking/investigating time relative to non-supplemented pigs. When supplementing RH to finishing pigs, Marchant-Forde et al. (2003) denoted an increase in pen activity by 19% . However, Marchant-Forde et al. (2008) and Athayde et al. (2013) concluded that BAA supplementation did not affect activity or movement in the pen, respectively. In rats, BAA (salbutamol, clenbuterol, isoproterenol and zinterol) have been shown to decrease locomotor behavior (O'Donnell, 1993a; O'Donnell, 1993b; O'Donnell, 1993c).

**Mobility.** Burson et al. (2014) concluded that supplementing ZH resulted in a tendency for a treatment  $\times$  time interaction in the travel speed of cattle from their home pens to the working facilities. In that study, the ZH supplemented cattle traveled significantly slower on d 0 and tended to travel slower on d 20 (no differences at d 5, 10, or 15 of the treatment period). No treatment had been applied at the d 0Z of the study, so the statistical difference on that day is likely due to random chance or sampling technique (Burson et al., 2014). The tendency at d 20Z does correspond with anecdotal reports that ZH supplemented cattle move slower during shipping. The cattle in Burson et al. (2014) were also considerably more aggressive and traveled more than twice the speed of the steers in other studies. As previously mentioned, the cattle in Burson et al. (2014) had minimal exposure to humans and the weighing process. Burson's sampling technique was also unique, as the cattle were all removed from their home pen and had to cross a fixed point in the alley before the timer was started. Samuelson et al. (2014) concluded that ZH supplementation did not affect speed of movement from the steer's home pen to a scale platform, but the technique utilized in that experiment was not described. Marchant-Forde et al. (2003) determined that pigs supplemented with RH took 136% longer to be removed from their home pen. It has also been determined that RH fed pigs require 24% more physical contact to be driven through an alley during loading (Rocha et al., 2013). Contradictorily, Marchant-Forde et al. (2008) concluded that supplementing R-salbutamol had no negative effects on moving pigs out of their home pen or into a weighing crate. In that same study, physical interaction was increased over time but not affected by treatment. Burson et al. (2014) reported that ZH supplementation did not affect travel speed between treatments when returning to their home pen, but cattle on both treatments did return home at a quicker rate. Marchant-Forde et al. (2008) agreed that BAA had no effect on the physical interactions or time required to return pigs to their home pens.

Hyperuricemia can lead to a type of arthritis known as gout. In humans, gout is a condition that is the result of needle-like crystals of uric acid accumulating in joints that cause pain and discomfort. A study in the swine industry concluded that BAA had no detrimental effects on joint

cartilage (He et al., 1992). The authors performed visual scores of cartilage and measured uronic acid concentrations of weight-bearing areas of the humeral and femoral condyles. No differences were determined for those parameters due to treatment.

Burson et al. (2014) utilized a 1 to 4 scoring system to assign mobility scores and concluded no differences between treatments in the percentage of normal (1 and 2's) and abnormal (3 and 4's) cattle. Cull Holstein cows were fed an 86% concentrate diet the final 90 d prior to harvest (Allen et al., 2009). When half of those cows were supplemented with  $312 \text{ mg} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$  of RH the final 32 days on feed, treatment had no effect on locomotion score. Supplementation of ZH to market dairy cows also had no effect on locomotion score (Lowe et al., 2012). Poletto et al. (2009) analyzed hoof lesions in pigs supplemented with and without RH. In that study, RH supplemented pigs had nearly twice as many hoof lesions (sand cracks, erosions and bruises) as control pigs. The authors stated that only three pigs were therapeutically treated for lameness during the entire study and two of those were control pigs. Supplementing salbutamol has increased the frequency and severity of hoof lesions in swine (Penny et al. 1994). These authors concluded that salbutamol may be interfering with horn production causing animals to be more vulnerable to hoof damage. Penny et al. (1994) also concluded that even though hoof lesions were more frequent with salbutamol supplementation, no differences were denoted in lameness between treatments.

### ***Effects on Heat Stress***

When supplementing ZH to conventionally fed steers and heifers during the fall, winter and spring, Wahrmond et al. (2014) reported that body temperature (measured by ruminal bolus) was not affected by treatment during the ZH feeding period. Body temperature averaged from 39.4 to 39.7°C for various groups of cattle, but those cattle were not finished during the heat of the summer. During the summer, rectal temperature has proven to increase by 0.7°C compared to during the winter (Gaughan et al., 2005). Burson et al., 2014 reported that rectal temperatures were greater in ZH supplemented versus non-supplemented steers during the summer, but the 0.02°C increase may not be

biologically significant. In those cattle, panting score was not affected by ZH supplementation, but it is important to note that these cattle were only subject to moderate heat stress. Macias-Cruz et al. (2010) determined that ZH supplementation to hair sheep during heat stress conditions increased the belly and flank skin surface temperature by greater than 4°C (35.1 vs. 39.5°C). The authors suggested this may be partially due to ZH altering microbial fermentation, ruminal digestion and intestinal environment.

### ***Effects on Animal Health***

Loneragan et al. (2014) concluded that mortality in feedlot cattle is rare (less than 0.5% during the final 24-29 d on feed), but administering BAA does increase the risk of death. These conclusions were drawn from large data sets (totaling more than 950,000 head), but it is important to note that these were retrospective analyses of mostly observational and unequally represented data sets. One data set was from a set of controlled, randomized studies, but they were not designed to investigate mortality. The authors also mention that there may be an unknown, confounding reason why a portion the cattle from the observational data sets were not fed a BAA. Perhaps they were targeting a value-added program or the cattle were projected to be too heavy if fed a BAA. Finally, the authors hypothesize that perhaps it is the change in management and feeding that is required during the BAA period that negatively affects cattle and is not an affect directly related to the feed additives. Large-scale, controlled and randomized experiments need to be conducted to fully investigate these potential concerns.

Overall, the effects on BAA on cattle health have been minimally investigated, especially in regard to the two BAA that are currently approved for use in the United States. Clenbuterol administration for 28 d had no effect on hemoglobin concentration or percentage of hematocrit in calves (Bruckmaier and Blum, 1992). Supplementation of ZH to finishing steers and heifers did not affect hematocrit percentage (Burson et al., 2014). Li et al. (2000) concluded that BAA (L-646,969) alone had no effect on leukocyte profile or lymphocyte function when supplemented to finishing

lambs for four weeks. Burson et al. (2014) reported no effects of ZH on blood pH; however, the authors reported that ZH supplementation increased potassium concentrations, but reduced calcium concentrations which would indicate that ZH caused a biologically significant difference in the cation-anion exchange.

Bruckmaier and Blum (1992) supplemented clenbuterol to calves for 35 days and found no differences in glucose concentration and a 33% decrease in lactate concentrations. Hansen et al. (1997) saw no effect of salbutamol supplementation on circulating glucose concentrations of pigs. Supplementing BAA have been extremely consistent in decreasing blood urea nitrogen concentrations in beef calves and sheep (Bruckmaier and Blum, 1992; Lopez-Carlos et al., 2012). The fact that implants and BAA decrease circulating urea nitrogen concentrations can be explained by the mode of action of these products, which promote muscle protein synthesis and utilization of nitrogen (Johnson and Chung, 2007).

Marchant-Forde et al. (2012) concluded that salbutamol had no effect on the percentage of pneumonia at harvest in palpated pig lungs. The BAA clenbuterol and cimaterol have been reported to induce hypertrophy of cardiac muscle and thus increase heart weight in rats and mice (Petrou et al., 1995; Eisen et al., 1988). Alternatively, salbutamol did not affect absolute heart weight or heart weight in relation to BW in pigs (Marchant-Forde et al., 2012; Hansen et al., 1997) or lambs (Sota et al. 1995). Burson et al. (2014) reported that ZH had no effect on the histologic results of heart tissue.

## **ROLE OF TECHNOLOGY IN SUSTAINABLE BEEF PRODUCTION**

The global population is predicted to exceed 9.1 billion people by the year 2050. This population growth and a growing “middle class” will equate to a 70 percent increase in demand for meat, milk, and eggs (FAO, 2010). This creates opportunities and considerable challenges for the U.S. livestock industry. The challenges come with the expectations of meeting these demands while using fewer inputs as competition for land, water, and energy intensifies. With these demands in mind, the goal of the beef industry seems relatively simple- become more efficient. However, the

beef industry is facing a challenge much more complicated than just efficiency; it is facing the challenges of sustainability. Sustainability can be defined in the beef industry as producing more beef in a manner that is profitable, environmentally friendly, and socially acceptable (Battagliese et al., 2013). In recent years, partial life cycle analyses have been conducted by using a deterministic model based on metabolism and nutrient requirements or the Integrated Farm System Model (IFSM) to determine the effect of technology use on sustainability. Utilizing all natural production systems instead of a conventional system that utilizes growth promoting technologies would require more land (12 to 22%), water (4 to 8%) and fossil fuels (8 to 17%), while producing more manure (10 to 23%) and greenhouse gases (10 to 17%; Capper, 2012; Capper and Hayes, 2012; Stackhouse et al., 2012). Currently, increasing animal productivity is believed to be one of the most effective mitigation strategies to decrease environmental impact. This strategy of “diluting” out maintenance cost is thought to be the most promising and sustainable mitigation approach to meet increasing demand for high quality protein. These same authors reported that a complete loss in technology use for the U.S. beef industry would result in an 8 to 8.5% increase in the cost of beef. This evidence may seem convincing, but these models have only accounted for two-thirds of the sustainability triangle. Social perception was not included. Currently, many consumers perceive the modern beef production system to have a far greater environmental impact than historical production systems. It is thought that increases in production efficiency have been achieved at the expense of environmental impact, animal health, animal welfare or product safety. A recent study by National Cattlemen’s Beef Association (NCBA) has provided more information on the beef industry’s contribution to sustainability. This full life cycle assessment by the IFSM included all three portions of sustainability and concluded that there has been a 5% improvement in sustainability from 2005 to 2011 (Battagliese et al., 2013). The positive effects of growth-promoting technologies on the environmental and economic pieces of sustainability have been recently documented, but more research is needed to determine the effects of these products on animal health and welfare. The beef industry needs to

proactively educate the general public about technology, how it is used and how it affects our industry, the animals and product safety.

### **CONCLUSIONS FROM LITERATURE REVIEW**

Growth-promoting technologies have been widely adopted in the North American feedlot industry as a method of improving performance, efficiency and total beef production. The production advantages of these products are well established, but the body of literature in regard to their effects on behavior, mobility, health, and heat stress of finishing steers is very limited. Increasing consumer concerns of product safety and animal welfare when these technologies are utilized has encouraged more research to be conducted in this area. In addition, recent anecdotal reports have suggested that some of these products (primarily ZH) may have negative impacts to animal health and welfare. To this point, the literature would suggest that these products have little to no effects on animal behavior and welfare, but more data is needed to validate these results in current commercial settings.

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## CHAPTER III

### EFFECTS OF GROWTH-PROMOTING TECHNOLOGIES ON TEMPERAMENT, BEHAVIOR AND MOBILITY OF FINISHING STEERS

**ABSTRACT:** Crossbred steers ( $n = 336$ ; initial BW =  $379 \pm 8$  kg) were utilized in a randomized complete block design to determine the effects of technology use in feedlot production systems on animal behavior and mobility. Treatments consisted of an all-natural treatment (receiving no growth promoting technologies; **NAT**), a conventional treatment (implanted with 40 mg of estradiol and 200 mg of trenbolone acetate [TBA] on d 0, and fed 33 and 9 mg/kg of monensin and tylosin daily, respectively; **CONV**) and a CONV treatment plus the addition of zilpaterol hydrochloride (ZH; at 8.33 mg/kg [90% DM basis] for the last 20 days on feed with a 3 to 4 d withdrawal prior to harvest; **CONV-Z**). Handling assistance, temperament and exit scores at the chute and temperament in each home pen were collected every 28 d until d 84, and then every 10 d during the ZH feeding period (denoted as d 0Z, 10Z and 20Z). Pen activity, step length and travel velocity was assessed during the ZH feeding period. Individual and group mobility was scored prior to loading at the feedlot and while unloading at the abattoir. There was a treatment  $\times$  time interaction for chute temperament score ( $P = 0.02$ ), with NAT steers being more restless than CONV steers at d 56 (2.24 vs. 1.98;  $P = 0.02$ ). Chute exit scores resulted in a treatment  $\times$  time interaction ( $P = 0.03$ ), with NAT steers having a more aggressive exit score than CONV and CONV-Z steers at d 10Z (2.18 vs. 1.89 and 1.88;  $P = 0.04$ ) and d 20Z (2.24 vs. 1.93 and 1.86;  $P = 0.03$ ). There were no effects of treatment on exit velocity, pen temperament, or overall temperament ( $P \geq 0.26$ ). Standing time and lying bouts were not affected by treatment ( $P > 0.45$ ), but CONV-Z steers took more steps/d (1,382 vs. 1,001;  $P = 0.04$ ), resulting in a

greater motion index (5608 vs. 4049;  $P = 0.05$ ) than NAT steers. While moving to the working facilities, CONV-Z steers moved at the slowest velocity, CONV were intermediary, and NAT the fastest (0.76 vs. 0.88 vs. 0.96 m·s<sup>-1</sup>;  $P < 0.05$ ). Step length was not affected by treatment at d 0Z or d 20Z ( $P \geq 0.38$ ). Treatment had no effect on individual or group mobility score ( $P \geq 0.14$ ), but a greater percentage of steers received an “abnormal” mobility score at the abattoir than at the feedlot (5.4 vs. 1.0%;  $P = 0.02$ ). The results of this experiment suggest that growth promoting technologies have little to no overall effects on cattle behavior and mobility. Thus, conventionally utilized technologies did not have a negative effect on finishing steer well-being during this study.

**Keywords:** beef cattle, behavior,  $\beta$ -adrenergic agonist, conventional, mobility, natural

## INTRODUCTION

Combination implants (containing estradiol and trenbolone acetate) and beta-adrenergic agonists (BAA; ractopamine hydrochloride; and zilpaterol hydrochloride) are all United States FDA approved products for use in feedlot cattle to improve growth efficiency and lean tissue gain. As of 2000, more than 90% of US feedlot cattle were receiving some type of steroidal implant (NAHMS, USDA 2000); and as of August 2013, approximately 60-70% were receiving some type of BAA. As a result of improved production efficiency, utilizing these technologies can have multiple benefits: increased beef supply, reduced cost to consumers, reduced usage of natural resources, and reduced greenhouse gas production (Avery and Avery, 2007; Lawrence and Ibarburu, 2006). Ractopamine hydrochloride (RH) is also utilized in the swine industry for increased efficiency and carcass leanness, but inconsistent results indicate that RH may negatively influence mobility, behavior, and susceptibility to handling and transportation stress in swine (Marchant-Forde et al., 2003).

Power of Meat (2014), reports that natural/organic beef sales have increased 2.6% versus a year ago, while total pounds of beef sold have decreased 1.2%. The consumer demand for natural/organic products has been rapidly increasing the past 5 years. When surveyed, one of the

consumer's top responses (fifth overall) for purchasing natural/organic products was "perceived benefits in animal welfare" (Power of Meat, 2013).

Extensive research has been conducted examining the effects of growth implants, BAA and other technologies such as ionophores on feedlot performance and carcass characteristics. However, there is a limited amount of research relative to the effects of these technologies on animal behavior and welfare in feedlot cattle. To the authors' knowledge, no published studies have analyzed the effects of natural and conventional production systems with today's current technologies on behavior and mobility in feedlot steers. As a result, the objectives of this study were to examine the effects of conventional beef production systems with and without the use of a BAA on behavior and mobility compared to an all-natural production system.

## **MATERIALS AND METHODS**

All protocols were approved by the Oklahoma State University Institutional Animal Care and Use Committee.

### ***Cattle Management***

A more detailed description of cattle management in this experiment is published elsewhere (Maxwell et al., 2014). In April of 2013, 423 black-hided certified natural steers were transported from Willow Lake, SD (n = 303), and Cedar Rapids, NE (n = 120) to the Willard Sparks Beef Research Center, Stillwater, OK. On May 01 and May 03, 2013, the cattle from South Dakota and Nebraska were processed and 87 steers were sorted off due to weight and utilized in a different experiment. A total of 336 steers were enrolled in this experiment on 3 different dates, May 07, 09 and 23, 2013. Animal behavior (assistance required to enter the chute, chute temperament score, exit score, and exit velocity) was measured at arrival and initial sorting to validate data collection procedures and to expose cattle to handling methods for this experiment. Steers were weighed, and chute temperament score, exit score, and exit velocity were obtained on d -1. The cattle were blocked

by BW within source and stratified by initial temperament measurements (chute temperament score, exit score, and exit velocity) and randomly allocated to study pens. On d 0, all cattle were weighed, and sorted to study pens (8 blocks; 1 replication/block; 8 pens/treatment; 14 steers/pen; 112 steers/treatment; initial BW =  $379 \pm 8$  kg). Treatments consisted of an all-natural treatment (NAT), a conventional treatment (CONV), or a conventional treatment with the addition of a beta-agonist at the end of the feeding period (CONV-Z). The NAT cattle received no antimicrobial, growth-promoting implants, or beta-agonists. The CONV and CONV-Z cattle were implanted with 40 mg of estradiol and 200 mg of trenbolone acetate (Revalor-XS<sup>®</sup>, Merck Animal Health) on d 0. They were also fed 33 and 9 mg/kg (DM basis) of monensin and tylosin (Rumensin<sup>®</sup> and Tylan<sup>®</sup>, Elanco Animal Health, Greenfield, IN) daily, respectively. The CONV-Z cattle were fed zilpaterol hydrochloride (ZH; Zilmax<sup>®</sup>, Merck Animal Health) at 8.33 mg/kg (90% DM basis) for the last 20 d on feed, and ZH was withdrawn from feed for 3-4 d prior to harvest. All cattle were fed the same base 93% concentrate diet as detailed by Maxwell et al. (2014). Briefly, the diet consisted of approximately 48% dry-rolled corn, 15% dried distiller grains, 15% wet-corn gluten, 15% supplement (liquid and dry), and 7% switchgrass hay. All diets were formulated to meet or exceed NRC (2000) requirements.

The same personnel were designated the same tasks, including cattle handling, on each weigh day throughout the experiment. Certain technicians were assigned the responsibility of evaluating specific subjective scores. The same technician consistently assigned the same subjective scores on every collection d. Assistance required to enter the chute, chute temperament score, exit score, and exit velocity were collected on every weigh day; while pen temperament scores were collected the day after cattle were weighed starting on d 29. Cattle were weighed on d 0, 28, 56, and 84 of the finishing phase. On d 84, blocks of cattle were projected into slaughter groups based upon projected slaughter BW and a visual appraisal of 12<sup>th</sup> rib-fat thickness. On August 19 and 20, 2013, d 103 and 104, respectively, all cattle except for the 2 lightest blocks were weighed and the CONV-Z cattle were started on ZH. The light 2 blocks were weighed and the CONV-Z started on ZH on October 08, 2013 (d 138). This date is referenced as d 0Z, the cattle were then weighed on d 10Z, and d 20Z.

Velocity traveling from and returning to the steers home pen and stride length was measured during the ZH feeding period. Pedometers (capable of measuring standing time, lying bouts and number of steps taken) were placed on 2 steers and accelerometers (capable of measuring standing time and lying bouts) were placed on 4 steers per pen (18 pens total) from d 0Z to d 20Z. Cattle on CONV-Z were fed ZH at a rate of  $87.6 \text{ mg} \cdot \text{steer}^{-1} \cdot \text{d}^{-1}$  based upon calculated intake and assayed zilpaterol values with a 3-4 d period of ZH withdrawal.

Cattle were fed for an average of 136 days. The cattle were slaughtered in two separate groups. The first group (6 blocks) was slaughtered on September 12 and 13, 2013, and the second group (2 blocks) was slaughtered on October 31 and Nov 01, 2013. All cattle were shipped 115 km to Creekstone Farms, Arkansas City, KS for slaughter. The CONV and CONV-Z cattle were slaughtered on the respective Thursdays, and the NAT cattle were slaughtered on the Fridays of each harvest week. This difference in ship date was due to the requirements of the packing facility in that they only slaughter NAT cattle on Friday of each week. Individual cattle mobility was assessed at the end of the ZH feeding period and mobility scores were assigned to groups of steers (approximately 16 hd) while the steers were being loaded at the feedlot and unloaded at the abattoir.

### ***Cattle Behavior***

Cattle were subject to routine handling and behavior data collection three times over a ten day period prior to the initiation of this trial to try to ensure these experiences were not completely novel events. When the rear gate of the chute was opened each steer was allowed five seconds to enter the chute on their own (note the steer would be in front of an alley stop and an individual would be standing outside the alley at the rear of the animal). If the steer did not enter or make an attempt to enter the chute in the allotted time, the individual at his rear would utilize vocal and physical (pat the steer on the hip with his hand) encouragement for 5 seconds. If the steers still refused to enter the chute, the employee would locate an electrical prod and encourage the steer by a single placement of the prod to the hind quarter of the animal. The electrical prod was utilized as a “last resort” and very

seldom was discharged more than once. These data quantified the percentage of cattle that needed assistance entering the chute.

One observer, blinded to the treatments, scored each steer for chute temperament on a four-point scale (Grandin, 1995). The observer watched the steer for 10 seconds after the steer's head was caught in the head gate and was restrained by the squeeze chute. The scores were: 1 = calm, no movement; 2 = restless, shifting weight; 3 = head throwing, squirming and occasionally shaking the squeeze chute; 4 = violently and continually shaking the squeeze chute.

Exit score was assessed by the same observer on a four-point scale as the steers exited the squeeze chute (Lanier and Grandin, 2003). The scores were: 1 = walk; 2 = trot; 3 = run; 4 = jump. Exit velocity utilized infrared sensors (Polaris Multi Event Timers, Farmtek, Inc., Wylie, TX) to determine the time taken for an animal to transverse a fixed distance of 1.83 m after exiting the squeeze chute (Burrow et al., 1988).

Pen temperament was assessed by a single observer prior to the morning feeding on the day following each weigh day. This observation was initiated on day 29 to allow cattle to become acclimated to their pen mates and environment. The technician would enter the pen from the center of the feed bunk and walk approximately 10 m into the pen and then stop for 10-15 s. The technician would then walk approximately 10 more m into the pen and stop for 10-15 s. If needed, animals along the perimeter of the pen would be approached at a walk to observe their response to human interaction. The technician was continually observing all animals in the pen while walking and when stopped. The steer's responsiveness was scored on a five-point scale: 1 = nonaggressive (docile) – walks slowly, can approach closely, not excited by humans or facilities; 2 = slightly aggressive – walks quickly or trots away, carries head up at attention, maintains distance as human approaches; 3 = moderately aggressive – trots or runs along fences, head high and aware of humans, will move quickly as humans move closer, commonly separates themselves from the group; 4 = aggressive – runs, stays in the back of group, head high and very aware of humans, may run into fences and gates even with some distance, will likely run along fences if alone in pen; 5 = very aggressive – excited,

runs into fences, runs over humans and anything else in path, “crazy” (Hammond et al., 1996).

Additionally, an overall temperament score was calculated by averaging the steers chute temperament score ( $\frac{1}{3}$ ), exit score ( $\frac{1}{3}$ ) and pen temperament ( $\frac{1}{3}$ ).

Pedometers (IceQube, IceRobotics, Edinburgh, UK) were strapped on the right hind leg (around the mid-lower metatarsus) of two steers per pen during the ZH feeding period. The activity monitors use 3-axis accelerometer technology to measure time spent standing and lying, frequency of lying bouts, step counts, and motion index (a 3-dimensional proprietary measure of activity). During this same period, accelerometers (Onset, Pendant G Data Loggers, Pocasset, MA), inside protective capsules, were strapped to the right hind leg (around the mid-lower metatarsus) of four different steers per pen to measure standing and lying activity. Due to limited numbers, pedometers and accelerometers were only utilized on cattle in six of the eight weight blocks ( $n = 6$ ; 4 blocks from the first harvest and 2 from the second harvest).

### ***Cattle Mobility***

During the ZH period, the time for a pen of steers to travel from their home pen to the working facilities and from the working facilities to their home pen was recorded. This time, as well as the distance from each home pen to the working facilities, was utilized to calculate the velocity ( $\text{m}\cdot\text{s}^{-1}$ ) of travel from and to the steer’s home pen. Steers were moved by a single technician, on horseback, throughout the entire study. Traveling to the working facilities, the technician would start timing when he opened the gate to enter the steer’s home pen and would stop when the last steer from that pen traversed a fixed point at the working facilities. Traveling to their home pen, the technician would start timing when he opened the gate of a holding pen at the working facility and would stop when the last steer entered his home pen.

Whole blood samples ( $\sim 10$  mL) were collected into blood tubes containing no additives on d 0, 56, 0Z, 10Z, and 20Z. Whole blood was allowed to clot for 24 h at  $4^{\circ}\text{C}$  and serum was collected

after centrifugation at  $2,500 \times g$  for 20 min at 4°C. Serum was stored at -20°C until analyzed for uric acid (Biolis24i Chemistry Analyzer, Carolina Liquid Chemistries Corp., Winston-Salem, NC).

When the cattle were exiting the chute during the ZH period, a video camera (Axis P1353-E, Axis Communications, Lund, Sweden) recorded the cattle from a 90° angle as they were individually walking down an alley approximately 10 m from the chute. Still pictures were captured from these videos for quantification of step length. Step length was measured from the front of one rear hoof to the front of the other rear hoof when both hooves were in contact with the surface. This was quantified utilizing ImageJ software (<http://imagej.nih.gov/ij/>) to compare the distance between the two rear hooves to the distance between two known reference points. These videos were also utilized for a technician to assign individual mobility scores on a four-point scale adopted from Lily Edwards-Callaway (JBS, Greeley, CO, personal communication). The scores were: 1 = normal – long, fluid stride, even rhythm, and weight bearing on all four feet; 2 = slightly hesitant and stiff, shuffles feet, but still moves with the herd; 3 = obviously stiff and sore-footed, reluctant to move, cannot keep up with the herd; 4 = extremely reluctant to move, animal refuses to move even when encouraged by a handler, any steps are short and very unsteady. Mobility scores (1 to 4 as previously described) were also assigned to cattle as groups of steers were being loaded at the feedlot and unloaded at the abattoir. A trained technician, blinded to the treatments, observed groups of steers as they traveled through a 3.66 m alley directly prior to loading onto a truck. At the abattoir, the same technician observed the cattle exiting the truck onto a flat, concrete unloading dock with deep, diamond grooves. The technician attempted to assign every animal a score, but due to the width of the alley, some steers moved in a tight group and were not able to be clearly observed. If the observer was not able to clearly evaluate a steer's movement that steer was not assigned a score. The number of “unscored” steers were recorded. All steers receiving a mobility score of 1 or 2 were classified as “normal” in their mobility and steers receiving a mobility score of 3 or 4 were classified as “abnormal”. All unscored steers were assumed to be “normal”. This decision was made since all “abnormal” steers by

definition would not have been able to keep up with the pace of a healthy herd and clearly would have been individually identified.

### ***Statistical Analysis***

All data were analyzed from a randomized complete block design, with pen considered the experimental unit and weight block included as a random effect. The percentage of cattle requiring assistance to enter the chute and percentage of “abnormal” mobility scores were analyzed utilizing PROC GLIMMIX (SAS 9.3; SAS Inst. Cary, NC). Mixed models repeated measures methods were used, and fit statistics were compared to determine covariance structure for variables measured over time. Differences were considered significantly different when  $P \leq 0.05$ , and a trend when  $0.05 < P \leq 0.10$ .

## **RESULTS**

For detailed feedlot performance and carcass characteristic results please refer to Maxwell et al., (2014). Briefly, the CONV cattle had a 32% increase in ADG and 26% improvement in efficiency versus the NAT cattle ( $P < 0.01$ ; data not shown). The CONV-Z cattle had a 34% increase in ADG and 33% improvement in efficiency versus the NAT cattle ( $P < 0.01$ ; data not shown).

### ***Cattle Behavior***

All cattle temperament data was averaged and summarized by pen. As anticipated, there were no differences on d 0 for any behavior measurement. The percentage of cattle requiring assistance to enter the chute was not affected by treatment, time, or the interaction of treatment  $\times$  time ( $P > 0.32$ ; Table 3.1). There was a treatment  $\times$  time interaction for chute temperament score ( $P = 0.02$ ; Table 3.1), with NAT cattle being more restless than CONV cattle at d 56 (2.24 vs. 1.98;  $P = 0.02$ ). The CONV-Z steers tended to be more restless than the CONV steers (2.14;  $P = 0.08$ ) on the same day. A tendency for a treatment effect was also detected on d 20Z, with the CONV-Z cattle

being calmer than the CONV cattle (1.86 vs. 2.07;  $P < 0.08$ ) and the NAT cattle intermediary (1.98). No differences were noted at any other time point. Chute exit scores resulted in a treatment  $\times$  time interaction ( $P < 0.03$ ; Table 3.2), with NAT cattle tending to have a greater exit score than CONV and CONV-Z cattle on d 0Z (2.34 vs. 2.08 and 2.07;  $P = 0.09$ ), on d 10Z (2.18 vs. 1.89 and 1.88;  $P = 0.04$ ), and on d 20Z (2.24 vs. 1.93 and 1.86;  $P < 0.03$ ). There were no effects of treatment on exit velocity ( $P = 0.62$ ; Table 3.2). Over time, exit velocity was significantly reduced ( $P < 0.01$ ) in all treatments, but the NAT steers were numerically greater than the CONV and CONV-Z steers toward the end of the feeding period. Pen temperament was not affected by treatment ( $P = 0.26$ ; Table 3.3), but steers in all treatments became less responsive to a human observer entering the pen over time ( $P < 0.01$ ). With minimal differences in chute score and exit score and no differences in pen temperament, the calculated overall temperament score was not affected by treatment ( $P = 0.39$ ; Table 3.3). Over time, steers in all treatments did become less temperamental ( $P < 0.01$ ).

Pen behavior and activity results are summarized in Table 3.4 as average steps or motion index  $\cdot \text{steer}^{-1} \cdot \text{d}^{-1}$ . No differences were detected between treatments for standing time or lying bouts during the ZH period ( $P > 0.45$ ). From d 0Z to d 10Z, the NAT steers took fewer steps per day than the CONV-Z steers (936 vs. 1,370 steps  $\cdot \text{d}^{-1}$ ;  $P = 0.04$ ) and the CONV steers were intermediary (1,186). This increase in number of steps taken resulted in an increased motion index for the CONV-Z versus the NAT steers (5,497 vs. 3,678;  $P = 0.05$ ) and the CONV steers were intermediary (4,765). A similar treatment effect was revealed from d 10Z to d 20Z, with the NAT steers taking fewer steps per day (1,063 vs. 1,393 steps  $\cdot \text{d}^{-1}$ ;  $P = 0.06$ ) and reporting a reduced motion index (4,404 vs. 5,715;  $P = 0.07$ ) compared to the CONV-Z steers. During the entire ZH period, CONV-Z steers took more steps (1,382 vs. 1,001;  $P = 0.04$ ) and had a significantly greater motion index (5,608 vs. 4,049;  $P = 0.05$ ) compared to the NAT steers. For both measurement the CONV steers were intermediate (1,206 and 4,997) and not different from other treatments ( $P > 0.10$ ).

### ***Cattle Mobility***

As the steers were moved from their home pen to the working facility during the ZH period, the CONV-Z steers moved at a slower velocity than the CONV steers (0.76 vs. 0.88 m·s<sup>-1</sup>;  $P < 0.01$ ; Table 3.5) and CONV steers moved at a slower velocity than the NAT steers (0.88 vs 0.96 m·s<sup>-1</sup>;  $P = 0.04$ ; Table 3.5). In general, steers in all treatments moved at a slower rate over time ( $P < 0.01$ ). Cattle velocity from the working facilities to their home pens were not affected by treatment ( $P = 0.19$ ). There was a time effect ( $P = 0.02$ ), with cattle velocity being reduced on d 10Z compared to d 0Z and d 20Z (1.03 vs 1.19 and 1.16 m·s<sup>-1</sup>). Steers did travel at a more accelerated rate when returning to their home pen versus traveling to the working facility (1.13 vs. 0.90 m·s<sup>-1</sup>;  $P < 0.01$ ).

Uric acid concentrations analyzed in serum yielded a treatment effect, with NAT cattle having lower concentrations than the CONV and CONV-Z steers (0.65 vs 0.7 and 0.7;  $P = 0.01$ ; Table 3.6). Concentrations of uric acid also increased over time for all treatments ( $P < 0.01$ ). Data collected for mobility analysis are presented in Table 3.7. Images analyzed for step length of steers resulted in no time or treatment differences at d 0Z or d 20Z ( $P \geq 0.38$ ). Steps were approximately 58.7 cm on d 0Z and 56.9 cm on d 20Z. On a scale of 1 to 4, the average individual mobility score for all treatments was 1.08 and resulted in no treatment effect on the day the steers were shipped to the abattoir ( $P = 0.93$ ). The percentage of abnormal steers (1.86%) was not affected by treatment on the day the steers were shipped ( $P = 0.88$ ). Individual cattle, when scored in a group, during loading and unloading were also classified as having a “normal” or “abnormal” mobility score as previously described. At loading, 1.0% were classified as abnormal and treatment had no effect on the percentage of steers with an abnormal mobility score ( $P = 0.99$ ). During unloading at the abattoir, a greater percentage of steers were classified as abnormal for all treatment versus at loading (5.4 vs. 1.0%;  $P = 0.02$ ), but no statistical differences were detected between treatments at unloading ( $P = 0.14$ ). The change in the percentage of abnormal steers from loading to unloading tended to be greater for the CONV-Z steers compared to the NAT steers ( $P = 0.06$ ), and intermediary for the CONV steers. The technician observing the steers during loading and unloading, also recorded the number of steers that were not assigned a mobility score. These steers were classified as “unscored”.

Approximately 28% of the steers were not assigned a mobility score during loading, with no differences between treatments ( $P = 0.61$ ). Significantly fewer steers were “unscored” during unloading at the abattoir, with the greatest percentage of steers being “unscored” in the CONV-Z treatment versus the NAT and CONV treatments (9.6 vs. 2.2 and 0.9%;  $P = 0.03$ ).

## DISCUSSION

Temperament has been defined as the behavioral responses of cattle when exposed to human interactions (Fordyce et al., 1988) or a stressful event (Café et al., 2011). Excitable temperaments have resulted in reduced performance, efficiency, and meat quality (Fordyce et al., 1998; Nkrumah et al., 2007). Extreme or reactive responses could also be detrimental to cattle welfare and the safety of human handlers. From an emotional or feeling-based perspective, good welfare is the maximization of positive emotions and reduction of negative ones (Duncan, 1996). Other studies in swine have analyzed the effects on anabolic growth promotants or beta-agonist on aggression in bulls and/or steers and the effects of beta-agonist on behavior and mobility. This study is one of the first to analyze the effects of multiple technology use on the behavior, mobility and observed welfare of steers in a detailed fashion.

### *Cattle Behavior*

Baszczak et al. (2006) reported no difference in entry force score (a measurement of assistant needed to load into the chute) when steers were fed with and without RH the final 28 days on feed. The current study also detected no difference between treatments for steers entering the chute. Based on the definitions of their scoring scale, the percentage of cattle requiring assistance to enter the chute in Baszczak et al. (2006) was similar to the 52% that required assistance to enter the chute in the current study. Baszczak et al. (2006) did conclude that the steers receiving a RH entered the chute at a quicker speed than non-supplemented steers, but this parameter was not measured in the current study. Angus and Limousin cattle spending 208 days of feed were implanted with 36 mg of zeranol

every 70 d. This implant strategy displayed no effect on the time for cattle to enter the chute compared to non-implanted counterparts (Vanderwert et al., 1985). Baker and Gonyou (1986) concluded that implanting (36 or 72 mg of zeranol) reduced the time it took cattle to enter the chute pre-castration, but post-castration there were no differences between implanted or non-implanted steers. Feeding BAA to pigs has yielded inconsistent results while loading pigs into a weighing scale. Marchant-Forde et al (2003) concluded that RH fed pigs required more time and more pats, slaps, and pushes from handlers to enter the weighing crate. Opposing results were concluded by Marchant-Forde et al. in 2008 when supplementing R-salbutamol to finishing pigs. That study resulted in no effect of treatment on the physical interactions needed to move the pigs onto a weigh scale.

On d 20Z of the current study, the CONV steers tended to be more aggressive than the CONV-Z steers while restrained in the chute. While Baszczak et al. (2006), reported no differences in chute temperament score between steers supplemented with and without RH. Based on the definitions, their steers acted calmer while experiencing light pressure in the chute, but a slightly different scoring scale was utilized and the steers were not secured in the chute's head catch as in the current study. Alternatively, Burson et al. (2014) concluded that cattle receiving ZH had elevated chute temperament scores on d 20Z. In that study a scoring scale of 1 to 5 was utilized and the cattle were also not restrained in the chute's head catch. Vanderwert et al. (1985) reported that implantation had no effect on scale temperament score. Baker and Gonyou (1986) agreed with this conclusion post-castration, but pre-castration increasing dosages of zeranol caused a linear increase in chute temperament. Implantation with zeranol versus estradiol-benzoate caused no differences in mean chute temperament scores (Stricklin et al., 1979).

Exit score was unaltered by BAA supplementation in the current study and as reported by Baszczak et al. (2006). However, the CONV and CONV-Z steers were less aggressive exiting the chute than NAT steers near the end of the feeding period. This equated to a 13 and 15% reduction in mean exit score on d 10Z and d 20Z, respectively. Burson et al. (2014) did not measure exit score, but the investigators did conclude a treatment  $\times$  time interaction for exit velocity, with ZH

supplemented cattle exiting  $0.45 \text{ m}\cdot\text{s}^{-1}$  faster on d 5 of the treatment period, but no differences at d 0, 10, or 20. This result was contradictory to the current study, where no differences were detected between treatments for exit velocity. The current study could have potentially missed the peak in exit velocity that was reported by Burson et al. (2014) because d 5 of the ZH period was not measured in the current study. This variation in data may also be contributed to initial and overall difference in cattle temperament and personnel. Burson et al. (2014) reported mean exit velocities from 2.9 to  $3.6 \text{ m}\cdot\text{s}^{-1}$ , while the current study reported 1.8 to  $2.0 \text{ m}\cdot\text{s}^{-1}$  during the ZH supplementation period. In the current study, the steers did become calmer over time as they were exiting the chute. The study performed by Burson et al (2014) was only a 23 d finishing period in which the cattle were handled minimally prior to the start on ZH supplementation. Perhaps the variation in study length and previous handling contributed to the variation in chute behavior and exit velocity results between these two studies. Time for cattle to exit the chute was recorded by Vanderwert et al. (1985) and Baker and Gonyou (1986). Neither study detected a treatment effect of implantation on the total time for cattle to exit the chute.

Pen temperament of the steers decreased over time, likely becoming accustomed to the observer, but was not different between treatments. These results are supported by Marchant-Forde et al. (2008) who concluded that BAA had no effect on behavior responses of pigs to human presence. In that study, all pigs were willing to spend similar amounts of time close and/or touching the human observer. In cattle, Vanderwert et al. (1985) and Baker and Gonyou (1986) analyzed flight distance using a method similar to the one used in the current study to analyze pen temperament. Both of these studies concluded that zeranol implantation had no effect of flight distance (approximately 1.4 and 1 m, respectively). Hawkins et al. (2005) reported positive effects on docility when implanting steers and bulls with 36 mg of zeranol. Utilizing chute temperament, exit score, and pen temperament to calculate an overall temperament score resulted in no differences in overall cattle temperament in the current study.

Very limited to no data has been published in regard to cattle behavior (standing time and lying bouts) and activity (steps taken) in their home pen as a result of technology use. This type of pen behavior has been previously quantified in cattle with less aggressive implant strategies and in the swine industry as it relates to supplementing BAA. These studies have primarily utilized scan sampling and video recording to observe pen activity. The current study utilized pedometers and accelerometers to quantify standing time, lying bouts, steps taken, and an overall motion index. The current study found no differences between treatments for standing time or lying bouts. Marchant-Forde et al. (2003), Marchant-Forde et al. (2008), and Athayde et al. (2013) all concluded that BAA supplementation had no effect on the time pigs spend standing. However, when supplementing 20 mg/kg of RH, Schaefer et al. (1992) determined that finishing pigs tended to spend less time standing than non-supplemented pigs. The lower doses of 10 and 15 mg/kg did not affect standing time relative to non-supplemented pigs. Young calves were subject to implantation with 36 mg of zeranol or not implanted. Once at the feedlot these cattle were observed for pen activity (time spent standing or resting) and previous implant status had no effect on pen activity (Hawkin et al., 2005). Unruh et al. (1986) utilized an activity score (a composite of activity, restlessness and movement) to quantify the behavioral effects of implanting cattle every 84 d from birth to slaughter with 36 mg of zeranol. Repeated implantation with zeranol did not affect overall activity. Stricklin et al. (1979) revealed that implantation with zeranol versus estradiol-benzoate and progesterone had no effect on time spend lying or standing. In this study, CONV-Z steers took more steps than NAT steers, which resulted in a greater overall motion index for the CONV-Z steers. Overall, supplementing ZH did not cause any differences in the number of steps taken or motion index compared to CONV steers. This study was not designed to analyze the activity differences during various times of the year or various environmental conditions, but the authors did notice steer behavior and activity differences between the first and second harvest groups. Steers in the first harvest (steers that were finished during late August and early September) spent more time standing but took fewer steps than steers in the second harvest group (finished in late October; data not shown). During the first harvest, treatment averages

were similar for steps taken and motion index. During the second harvest, the NAT steers were essentially unchanged in their activity, while the CONV steers were more active and the CONV-Z steers were the most active. The majority of the overall activity differences were due to increased activity during the second harvest group (for CONV and CONV-Z steers). The weather conditions were considerably cooler and more favorable during the second harvest, but the second harvest only represented half as many steers that were fitted with pedometers and accelerometers. No differences were detected in the number of walking observations (observers count walking events) during the sampling hours of 0900 to 1500 in cattle implanted with zeranol or estradiol-benzoate (Stricklin et al., 1979). When supplementing RH to finishing pigs, Marchant-Forde et al. (2003) denoted an increase in pen activity. However, Marchant-Forde et al. (2008) and Athayde et al. (2013) concluded that BAA supplementation did not affect activity or movement in the pen, respectively. Supplementation with RH has also been proven to decrease the walking activity of pigs in their home pens (Schaefer et al., 1992). In rats, BAA (salbutamol, clenbuterol, isoproterenol and zinterol) have been shown to decrease locomotor behavior (O'Donnell, 1993a; O'Donnell, 1993b; O'Donnell, 1993c). These swine and rat studies were performed in controlled environments, and to the author's knowledge, there is no published cattle data to compare to our results under various environmental conditions as it relates to pen activity and technology use.

It is important to note that research investigating cattle behavior as it relates to technology use is very limited and our evaluation techniques and measurements are still evolving. The majority of studies that have investigated the effects of implantation were conducted prior to FDA approval of combination implants. The implants (primarily zeranol) utilized in those studies bind to different receptors and have different mechanism of action relative to TBA/estradiol implants. Zeranol is a synthetic "estrogen-like" compound that can alter metabolism directly through cellular receptors or indirectly through increase production of growth hormones. Estradiol and TBA combination implants do not alter circulating growth hormone concentration, but still increase insulin-like growth factor-1 in circulation and at the tissue level, suggesting variation in mode of action when TBA is included.

Zeranol implants would be considered mild in their potency (based on growth responses and anabolic activity on the compound) for finishing cattle relative to the growth implants that are currently available. The Revalor-XS (40 mg of estradiol and 200 mg of trenbolone acetate) implant that was utilized in the current study would be considered an aggressive implant strategy for cattle spending 136 days on feed.

### ***Cattle Mobility***

When moving the steers from their home pens to the working facilities during the ZH feeding period, the CONV-Z steers did move  $0.12 \text{ m}\cdot\text{s}^{-1}$  slower than the CONV steers and the CONV steers moved  $0.08 \text{ m}\cdot\text{s}^{-1}$  slower than the NAT steers. The average distance from the steer's home pens to the working facilities was 191 m. On average, it required 34 additional seconds to move the CONV-Z steers compared to the CONV steers, and 18 additional seconds to move the CONV steers versus the NAT steers. If this pace was maintained, it would take an additional 180 seconds to move the CONV-Z steers 1000 m versus the CONV steers, and 94 additional seconds to move the CONV steers compared to the NAT steers that same distance. Burson et al. (2014) concluded that supplementing ZH resulted in a tendency for a treatment  $\times$  time interaction in the travel speed of cattle from their home pens to the working facilities. In that study the ZH supplemented cattle traveled significantly slower on d 0Z and tended to travel slower on d 20Z (no differences at d 5, 10, or 15 of the treatment period). Obviously, no treatment had been applied at the d 0 of the study, so that statistical difference is likely due to random chance or sampling technique (Burson et al., 2014). The tendency at d 20 does correspond with difference in travel velocities that were reported in the current study. The cattle in the Burson et al. (2014) study were also considerably more aggressive and traveled at more than twice the speed of the steers in the current study. As previously mentioned, the cattle in the Burson et al. (2014) study had significantly less exposure to humans and the weighing process relative to the current study. Burson's sampling technique was also different as the cattle were all removed from their home pen and had to cross a fixed point in the alley before the timer was

started. The technician in the current study included the time it took him to remove the cattle from their pen. Samuelson et al. (2014) concluded that ZH supplementation did not affect speed on movement from the steer's home pen to a scale platform, but the technique utilized was not revealed. In support of the current study, Marchant-Forde et al. (2003) determined that pigs supplemented with a RH required more time to be removed from their home pen and more time to move the pigs to the weighing scale. It has also been determined that RH fed pigs require more physical contact to be driven through an alley during loading (Rocha et al., 2013). Contradictorily, Marchant-Forde et al. (2008) concluded that supplementing R-salbutamol had no negative effects on moving pig out of their home pen or into a weighing crate. In that same study, physical interaction was increased over time but not affected by treatment. Cattle movement from the working facilities back to their respective home pens were not different between treatments. As the steers were returning to their home pens they moved significantly quicker than when leaving their home pens. Burson et al. (2014) agreed that ZH supplementation did not affect travel speed, and that cattle return home at a quicker rate. Marchant-Forde et al. (2008) agreed that BAA had no effect on the physical interactions or time required to return pigs to their home pens.

Hyperuricemia can lead to a type of arthritis known as gout. In humans, gout is a condition that is the result of needle-like crystals of uric acid accumulating in joints that cause pain and discomfort. In this experiment, the NAT steers did have reduced concentration of circulating uric acid during the ZH feeding period, but values for all treatments were within normal reference values for yearling steers (Doornenbal et al., 1988). A study in the swine industry has also concluded that BAA had no detrimental effects on joint cartilage (He et al., 1992). He et al. (1992) performed visual scores of cartilage and measured uronic acid concentrations of weight-bearing areas of the humeral and femoral condyles. No differences were determined for those parameters due to treatment.

Step length in beef steers receiving various growth promoting technologies has not been previously published. The current study concluded that treatment did not affect step length at d 0Z or d 20Z. Numerically, the step lengths were slightly shorter on d 20Z for all treatments compared to d

0Z. Considering the frame and size differences, these step lengths of approximately 58 cm are comparable to the 137 cm stride lengths (one stride is equal to two steps) in healthy dairy cows that was reported by Phillips and Morris (2000).

Treatment did not affect individual mobility scores captured at the end of the feeding period. These results are supported by Burson et al. (2014) who utilized a similar 1 to 4 scoring systems and concluded no differences between treatments in the percentage of normal (1 and 2's) and abnormal (3 and 4's) cattle. Cull Holstein cows were fed an 86% concentrate diet the final 90 d prior to harvest (Allen et al., 2009). When half of the cows were supplemented with  $312 \text{ mg} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$  of RH the final 32 days on feed, treatment had no effect on locomotion score. Supplementation of ZH to market dairy cows also had no effect on locomotion score (Lowe et al., 2012). Poletto et al. (2009) analyzed hoof lesions in pigs supplemented with and without RH. In that study, RH supplemented pigs had nearly twice as many hoof lesions (sand cracks, erosions, and bruises) as control pigs. Even with those results, the authors stated that only three pigs were therapeutically treated for lameness during the entire study and two of those were control pigs. Supplementing salbutamol has increased the frequency and severity of hoof lesion (Penny et al. 1994). These authors concluded that salbutamol may be interfering with horn production causing animals to be more vulnerable to hoof damage. Penny et al. (1994) also concluded that even though hoof lesions were more frequent with salbutamol supplementation, no differences were denoted in lameness between treatments. In the current study, steers were also individually scored as each group (approximately 16 hd) were moved from their holding pens, through a 3.7 m wide concrete alley on their way to the loading area. The steers were transported approximately 115 km to Creekstone Farms Premium Beef in Arkansas City, KS. Steers were also individually assigned a mobility score by the same technician as they were unloading at the abattoir onto a level, concrete platform with deep diamond grooves. It is important to note that approximately 28% during loading and 4% during unloading were not assigned a score because they were hidden by another steer from a clear view of the technician. This percentage was significantly greater during the loading process for all treatments as the steers were moving down the wide alley in

more of a group versus when unloading, where they came off the trailer one or two at a time. Treatment had no effect on the percentage of steers assigned a “normal” or “abnormal” mobility scores during loading. More steers were assigned an “abnormal” mobility score while unloading at the abattoir, but treatment had no effect on the mobility score during unloading. Even though these steers were hauled a relatively short distance, the process of trucking seems to have had a negative effect on steers mobility in all treatments in this study. After long distance ( $\geq 400$  km) hauls, surveyed truck drivers revealed a 0.01% increase in lameness of finished cattle at the abattoir during unloading than at the feedlot during loading (Gonzalez et al., 2012). Gonzalez et al. (2012) also revealed that the proportion of total compromised animals decrease with more years of truck driving experience. Pilcher et al. (2011) revealed that hogs hauled 1 h or less in smaller floor spaces ( $0.317$  to  $0.350$  m<sup>2</sup>/100 kg of BW) increased the frequency of stress indicators and nonambulatory, noninjured (nonambulatory due to something other than injury) pigs compared to pigs hauled longer times or in larger floor spaces. A survey in 2010, reported an increase in pig lameness at the plants when transported in smaller floor spaces ( $\leq 0.38$  m<sup>2</sup>/100 kg of BW) for a relative short journey time ( $\leq 2.5$  h; Kephart et al., 2010). The effects of trucking on animal welfare need to be investigated more thoroughly. This feat is very difficult from a logistics stand point to capture observations on large numbers of cattle at loading and unloading by a single observer or similarly trained group of observes.

## CONCLUSIONS

For an industry to remain sustainable it must meet some balance of profitability, environmental friendliness, and social acceptance (Coopridge et al., 2011). Beta-agonists, growth-promoting implants, and ionophores are all valuable technologies that help improve the gain and efficiency of beef production; which ultimately reduces the cost of beef to consumers. These technologies also have positive impacts on water and land utilization, while decreasing the carbon footprint (Capper, 2012; Stackhouse et al., 2012). The current question is, “Are these production and

environmental advantages accompanied with unintended negative effects on animal behavior and well-being?” The current study is one of the first to be published in peer-reviewed literature that address this question in finishing cattle and clearly a larger literature pool in this area is needed. We conclude from this experiment, that these growth promoting products do not negatively affect the behavior, mobility, or the overall observed well-being of finishing beef steers.

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Table 3.1. The effects of treatment on the percentage of cattle requiring assistance to enter the chute and chute temperament score of finishing steers.

Item,	Treatment <sup>1</sup>			SEM <sup>2</sup>	P - value		
	NAT	CONV	CONV-Z		Trt	Time	Trt*Time
Assistance Required <sup>3</sup> , %	47.7	53.9	48.9	3.3	0.40	0.32	0.44
d 0	47.9	54.6	54.4				
d 28	49.9	51.0	45.6				
d 56	48.1	46.6	49.2				
d 84	46.8	62.2	52.4				
d 0Z	48.6	50.5	42.5				
d 10Z	46.3	53.2	50.1				
d 20Z	46.3	58.6	47.8				
Chute Temperament <sup>4</sup>	2.07	2.09	2.08	0.05	0.89	<0.01	0.02
d 0	2.16	2.21	2.21				
d 28	2.13	2.10	2.13				
d 56	2.24 <sup>b</sup>	1.98 <sup>a</sup>	2.14 <sup>ab</sup>				
d 84	1.96	2.03	2.03				
d 0Z	2.07	2.20	2.09				
d 10Z	1.93	2.06	2.05				
d 20Z	1.98 <sup>yz</sup>	2.07 <sup>z</sup>	1.86 <sup>y</sup>				

<sup>a,b</sup>Means within a row without a common superscript differ ( $P \leq 0.05$ ).

<sup>y,z</sup>Means within a row without a common superscript differ ( $0.05 < P \leq 0.10$ ).

<sup>1</sup>Treatments include 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z).

<sup>2</sup>Standard error of the mean (n = 8).

<sup>3</sup>Percentage of steers requiring any type of assistance (verbal, physical, or electric prod) to enter the chute.

<sup>4</sup>Mean chute temperament score (1-4): 1 = calm, no movement; 2 = restless, shifting weight; 3 = head throwing, squirming and occasionally shaking the squeeze chute; 4 = violently and continually shaking the squeeze chute.

Table 3.2. The effects of treatment on exit score and exit velocity of finishing steers.

Item,	Treatment <sup>1</sup>			SEM <sup>2</sup>	P - value		
	NAT	CONV	CONV-Z		Trt	Time	Trt*Time
Exit Score <sup>3</sup>	2.26	2.10	2.12	0.83	0.29	<0.01	0.03
d 0	2.34	2.35	2.38				
d 28	2.44	2.31	2.50				
d 56	2.03	2.05	2.13				
d 84	2.24	2.06	1.98				
d 0Z	2.34 <sup>z</sup>	2.08 <sup>y</sup>	2.07 <sup>y</sup>				
d 10Z	2.18 <sup>b</sup>	1.89 <sup>a</sup>	1.88 <sup>a</sup>				
d 20Z	2.24 <sup>b</sup>	1.93 <sup>a</sup>	1.87 <sup>a</sup>				
Exit Velocity <sup>4</sup> , m·s <sup>-1</sup>	2.22	2.14	2.14	0.08	0.62	<0.01	0.77
d 0	2.39	2.39	2.42				
d 28	2.50	2.50	2.56				
d 56	2.44	2.44	2.46				
d 84	2.09	1.98	1.94				
d 0Z	2.04	1.91	1.89				
d 10Z	1.96	1.91	1.82				
d 20Z	2.13	1.84	1.90				

<sup>a,b</sup>Means within a row without a common superscript differ ( $P \leq 0.05$ ).

<sup>y,z</sup>Means within a row without a common superscript differ ( $0.05 < P \leq 0.10$ ).

<sup>1</sup>Treatments include 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z).

<sup>2</sup>Standard error of the mean (n = 8).

<sup>3</sup>Mean exit score (1-4): 1 = walk; 2 = trout; 3 = run; 4 = jump.

<sup>4</sup>Mean exit velocity (m·s<sup>-1</sup>) as the cattle transverse a fixed distance of 1.83 m after exiting the squeeze chute.

Table 3.3. The effects of treatment on pen temperament and overall temperament score of finishing steers.

Item,	Treatment <sup>1</sup>			SEM <sup>2</sup>	P - value		
	NAT	CONV	CONV-Z		Trt	Time	Trt*Time
Pen							
Temperament <sup>3</sup>	1.17	1.17	1.13	0.04	0.26	<0.01	0.79
d 28	1.22	1.21	1.16				
d 56	1.21	1.22	1.17				
d 84	1.19	1.22	1.14				
d 0Z	1.19	1.14	1.15				
d 10Z	1.13	1.12	1.10				
d 20Z	1.11	1.14	1.07				
Overall							
Temperament <sup>4</sup>	1.82	1.77	1.75	0.04	0.39	<0.01	0.25
d 28	1.93	1.88	1.93				
d 56	1.82	1.75	1.81				
d 84	1.80	1.77	1.72				
d 0Z	1.87	1.81	1.77				
d 10Z	1.75	1.69	1.68				
d 20Z	1.78	1.71	1.60				

<sup>1</sup>Treatments include 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z).

<sup>2</sup>Standard error of the mean (n = 8).

<sup>3</sup>Mean pen temperament score (1-5): 1 = nonaggressive (docile) – walks slowly, can approach closely, not excited by humans or facilities; 2 = slightly aggressive – walks quickly or trots away, carries head up at attention, maintains distance as human approaches; 3 = moderately aggressive – trots or runs along fences, head high and aware of humans, will move quickly as humans move closer, commonly separates themselves from the group; 4 = aggressive – runs, stays in the back of group, head high and very aware of humans, may run into fences and gates even with some distance, will likely run along fences if alone in pen; 5 = very aggressive – excited, runs into fences, runs over humans and anything else in path, “crazy”.

<sup>4</sup>Mean overall temperament score: an average of chute temperament score, exit score and pen temperament.

Table 3.4. The effects of treatment on daily pen behavior and activity of finishing steers<sup>1</sup>.

Item	Treatment <sup>2</sup>			SEM <sup>3</sup>	P-value <sup>3</sup>
	NAT	CONV	CONV-Z		
<b>d 0Z to d 10Z</b>					
Standing Time, h	11.7	11.6	11.9	0.4	0.66
Lying Bouts	12.5	12.9	13.3	0.6	0.44
Steps	936 <sup>a</sup>	1186 <sup>ab</sup>	1370 <sup>b</sup>	144	0.04
Motion Index	3678 <sup>a</sup>	4765 <sup>ab</sup>	5497 <sup>b</sup>	585	0.05
<b>d 10Z to d 20Z</b>					
Standing Time, h	11.5	11.2	11.4	0.5	0.80
Lying Bouts	14.1	14.5	13.5	0.7	0.66
Steps	1063	1242	1393	121	0.06
Motion Index	4404	5239	5715	527	0.07
<b>d 0Z to d 20Z</b>					
Standing Time, h	11.6	11.4	11.7	0.5	0.76
Lying Bouts	13.3	13.7	13.4	0.7	0.85
Steps	1001 <sup>a</sup>	1209 <sup>ab</sup>	1382 <sup>b</sup>	129	0.04
Motion Index	4049 <sup>a</sup>	4997 <sup>ab</sup>	5608 <sup>b</sup>	545	0.05

<sup>a,b</sup>Means within a row without a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>All data are summarized by day.

<sup>2</sup>Treatments include 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z).

<sup>3</sup>Standard error of the mean (n = 6). P-value is for overall ANOVA.

Table 3.5. The effects of treatment on travel velocity of finishing steers to and from the working facilities.

Item	Treatment <sup>1</sup>			SEM <sup>2</sup>	P - value		
	NAT	CONV	CONV-Z		Trt	Time	Trt*Time
Travel to Facilities <sup>3</sup> , m·s <sup>-1</sup>	0.96 <sup>c</sup>	0.88 <sup>b</sup>	0.76 <sup>a</sup>	0.12	<0.01	<0.01	0.47
d 0Z	0.99	0.89	0.83				
d 10Z	1.04	0.87	0.77				
d 20Z	0.98	0.94	0.84				
d 23/24Z	0.83	0.81	0.59				
Travel to Home Pen <sup>4</sup> , m·s <sup>-1</sup>	1.18	1.13	1.07	0.11	0.19	0.02	0.54
d 0Z	1.18	1.22	1.17				
d 10Z	1.09	1.00	1.01				
d 20Z	1.26	1.17	1.04				

<sup>a,b,c</sup>Means within a row without a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>Treatments include 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z).

<sup>2</sup>Standard error of the mean (n = 8).

<sup>3</sup>Mean velocity for a pen of steers to travel from their home pen to the working facilities (191 m).

<sup>4</sup>Mean velocity for a pen of steers to travel from the working facilities to their home pen (191 m).

Table 3.6. The effects of treatment on uric acid concentrations of finishing steers.

Item,	Treatment <sup>1</sup>			SEM <sup>2</sup>	P - value		
	NAT	CONV	CONV-Z		Trt	Time	Trt*Time
Uric Acid, mg/dL	0.65 <sup>a</sup>	0.70 <sup>b</sup>	0.70 <sup>b</sup>	0.02	0.02	<0.01	0.12
d 0	0.66	0.64	0.66				
d 56	0.64	0.67	0.65				
d 0 Z	0.67	0.78	0.75				
d 10 Z	0.64	0.73	0.72				
d 20 Z	0.64	0.70	0.72				

<sup>a,b</sup>Means within a row without a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>Treatments include 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z).

<sup>2</sup>Standard error of the mean (n = 8).

Table 3.7. The effects of treatment on step length and mobility of steers prior to loading at the feedlot and while unloading at an abattoir.

Item	Treatment <sup>1</sup>			SEM <sup>2</sup>	P-value <sup>2</sup>
	NAT	CONV	CONV-Z		
Step Length, cm					
d 0Z	59.4	58.8	57.9	1.3	0.38
d 20Z	57.2	56.7	56.9	0.8	0.86
Individual Mobility					
Mobility Score <sup>3</sup>	1.08	1.09	1.08	0.02	0.93
Abnormal Mobility <sup>4</sup> , %	1.48	1.77	2.33	1.30	0.88
Group Mobility, %					
Abnormal – Loading <sup>4,5</sup>	3.0	0.0	0.0	1.5	0.99
Abnormal – Unloading <sup>4,5</sup>	4.4	2.3	9.4	2.6	0.14

<sup>a,b</sup>Means without a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>Treatments include 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z).

<sup>2</sup>Standard error of the mean (n = 8 for step length and individual mobility; n = 4 for group mobility). P-value is for overall ANOVA.

<sup>3</sup>Mobility score (1-4): 1 = normal – long, fluid stride, even rhythm, and weight bearing on all four feet; 2 = slightly hesitant and stiff, shuffles feet, but still moves with the herd; 3 – obviously stiff and sore-footed, reluctant to move, cannot keep up with the heard; 4 – extremely reluctant to move, animal refuses to move even when encouraged by a handler, any steps are short and very unsteady.

<sup>4</sup>Steers receiving a mobility score of 1 or 2 were classified “normal” in their mobility and cattle receiving a mobility score of 3 or 4 were classified as “abnormal”.

<sup>5</sup>At loading (28%) and unloading (4%), steers that could not be clearly evaluate by the technician were not assigned a mobility score. By definition these steers were assumed to be normal in their mobility.

## CHAPTER IV

### EFFECTS OF GROWTH-PROMOTING TECHNOLOGIES ON HEALTH PARAMETERS OF FINISHING STEERS

**ABSTRACT:** Crossbred steers ( $n = 336$ ; initial BW =  $379 \pm 8$  kg) were utilized in a randomized complete block design to determine the effects of technology use in feedlot production systems on health parameters of finishing steers. Treatments consisted of an all-natural treatment (defined as receiving no growth promoting technologies; **NAT**), a conventional treatment (implanted with 40 mg of estradiol and 200 mg of trenbolone acetate on d 0, and fed 33 and 9 mg/kg [DM basis] of monensin and tylosin daily, respectively; **CONV**) and a CONV treatment plus the addition of zilpaterol hydrochloride (**ZH**; at 8.33mg/kg [90% DM basis] for the last 20 days on feed with a 3 to 4 d withdrawal; **CONV-Z**). Steers were observed daily for signs of respiratory disease, lameness and any other abnormality. Blood samples were collected every 28 d until d 84, and then every 10 d during the ZH feeding period (denoted as d 0Z, 10Z and 20Z) to determine hematology. Blood pH and additional metabolites were determined during the ZH feeding period. At harvest, livers were observed for abscesses, lungs were palpated for abnormalities and liver and heart samples were collected for histology. All blood analytes affected by treatment were within clinically normal concentrations. There was a treatment  $\times$  time interaction for total white blood cells (WBC;  $P < 0.01$ ) with CONV and CONV-Z cattle having greater WBC counts than NAT cattle from d 28 (9.83 and 9.54 vs.  $8.60 \times 10^6/\mu\text{L}$ ) through d 20Z (10.83 and 11.25 vs.  $9.83 \times 10^6/\mu\text{L}$ ;  $P \leq 0.07$ ). Neutrophils counts were also greater for CONV and CONV-Z compared to NAT cattle from d 28 (2.57 and 2.47 vs.  $1.99 \times 10^3/\mu\text{L}$ ) through d 20Z (3.51 and 3.47 vs.  $2.44 \times 10^3/\mu\text{L}$ ;  $P < 0.05$ ). Neutrophil-to-Lymphocyte ratio was increased (0.54 and 0.52 vs. 0.4;  $P < 0.01$ ) and more monocytes were detected

in the CONV and CONV-Z cattle compared to the NAT cattle (1.21 and 1.22 vs.  $1.08 \times 10^{-3}/\mu\text{L}$ ;  $P < 0.01$ ). The CONV-Z cattle had reduced lactate concentrations compared to NAT and CONV cattle at d 10Z (13.5 vs. 28.9 and 27.3 mg/dL) and 20Z (12.5 vs. 25.1 and 27.2 mg/dL;  $P < 0.01$ ). Conventional cattle exhibited greater glucose concentrations than NAT and CONV-Z cattle (88.9 vs. 83.9 and 82.5 mg/dL;  $P < 0.01$ ). The CONV-Z cattle exhibited the greatest potassium concentrations ( $P < 0.01$ ). There was no significant effect of treatment on liver abscesses ( $P = 0.74$ ), lung scores ( $P > 0.09$ ) or liver and heart histologic changes ( $P \geq 0.45$ ). Collectively, this experiment demonstrates that growth promoting technologies did not affect overall health of finishing steers.

**Keywords:** beef cattle, blood metabolites,  $\beta$ -adrenergic agonist, conventional, health, natural

## INTRODUCTION

In an effort to meet growing protein demands and offset a dwindling U.S. cow herd, the U.S. beef industry has rapidly adopted FDA approved growth promoting technologies (i.e. growth implants and beta-adrenergic agonist; BAA) to produce more beef for human consumption. These technologies improve production efficiency, reduce the cost of beef for consumers (Lawrence and Ibarburu, 2006), improve water and land utilization, and decrease the carbon footprint (Capper, 2012; Stackhouse et al., 2012). Feedlot performance and carcass characteristics have been well documented with these products. However, there is limited research that has analyzed the effects of these technologies on animal health.

Administering exogenous growth promoting hormones in the form of implants may impact the ability of the immune system to respond to stressors because actions of the anabolic hormone increase protein anabolism and modify metabolism to enhance growth factors in exchange for energy and protein required for immune responses (Richeson et al., 2013). Supplementation of BAA can shift additional nutrients toward protein deposition and away from other metabolic pathways,

potentially further impacting immune responses. Stressful events lead to increased cortisol production, and though much emphasis has been placed on cortisol's immunosuppressive properties, it is clear that cortisol in moderation can also facilitate a positive immune response (Yeager et al., 2011). Considering this fact, it is crucial to consider that growth promoting implants have been proven to significantly moderate cortisol production, potentially promoting immune function.

The death of finishing feedlot cattle is a rare event, but recent anecdotal reports have generated concern that growth-promoting technologies (specifically BAA) may be linked to increases in cattle morbidity and mortality (Loneragan et al., 2014). To date, the body of literature is limited, so the objective of this study was to examine the effects of conventional beef production systems with and without the use of a BAA on the health of finishing steers compared to an all-natural production system.

## **MATERIALS AND METHODS**

All protocols were approved by the Oklahoma State University Institutional Animal Care and Use Committee.

### ***Cattle Management***

A more detailed description of cattle management in this experiment is published elsewhere (Maxwell et al., 2014b). In April of 2013, 423 black-hided certified natural steers were transported from Willow Lake, SD (n = 303), and Cedar Rapids, NE (n = 120) to the Willard Sparks Beef Research Center, Stillwater, OK. After processing, 87 steers were sorted off based on weight and assigned to another experiment. A total of 336 steers were started on this experiment on 3 different dates, May 07, 09 and 23, 2013. Steers were weighed on d -1, blocked by BW within source and allocated to study pens. On d 0, all cattle were weighed, and sorted to study pens (8 blocks; 1 replication/block; 8 pens/treatment; 14 steers/pen; 112 steers/treatment; initial BW =  $379 \pm 8$  kg).

Treatments consisted of an all-natural treatment (NAT), a conventional treatment (CONV), and a conventional treatment with the addition of a beta-agonist at the end of the feeding period (CONV-Z). The NAT cattle received no antimicrobials, growth-promoting implants, or beta-agonists. The CONV and CONV-Z cattle were implanted with 40 mg of estradiol and 200 mg of trenbolone acetate (TBA; Revalor-XS<sup>®</sup>, Merck Animal Health, DeSoto, KS) on d 0. They were fed 33 and 9 mg/kg (DM basis) of monensin and tylosin (Rumensin<sup>®</sup> and Tylan<sup>®</sup>, Elanco Animal Health, Greenfield, IN) daily, respectively. The CONV-Z cattle were fed zilpaterol hydrochloride (ZH; Zilmax<sup>®</sup>, Merck Animal Health) at 8.33 mg/kg (90% DM basis) for the last 20 d on feed, and ZH was withdrawn from feed for 3 to 4 d prior to slaughter. All cattle were fed the same base 93% concentrate diet as detailed by Maxwell et al. (2014b). On a DM basis, the diet consisted of approximately 48% dry-rolled corn, 15% dried distiller grains, 15% wet corn gluten, 15% supplement (liquid and dry), and 7% switchgrass hay. All diets were formulated to meet or exceed NRC (2000) requirements.

Six steers per pen that represented the median BW of that pen were selected on d -1 as a pen subset. Blood samples were collected from this subset of steers plus one alternate steer (n = 7) on every weigh day and those dates were d 0, 28, 56, and 84 of the finishing phase. On d 84, blocks of cattle were projected into harvest groups based upon projected harvest BW and a visual appraisal of 12<sup>th</sup> rib-fat thickness. On August 19 and 20, 2013, d 104 and 103, respectively, all cattle except for the 2 lightest blocks were weighed and the CONV-Z cattle were started on ZH. The 2 lightest blocks were weighed and the CONV-Z steers started on ZH on October 08, 2013 (d 138). The initial d of ZH supplementation is referenced to as d 0Z (d 138) and spans through d 20Z (d 158). The cattle were also weighed on d 10Z and d 20Z. Cattle on CONV-Z were fed Zilmax at a rate of 87.6 mg·steer<sup>-1</sup>·d<sup>-1</sup> based upon calculated intake and assayed zilpaterol values with a 3-4 d period of ZH withdrawal.

The cattle were harvested in two separate groups. The first group (6 blocks) was slaughtered on September 12 and 13, 2013, and the second group (2 blocks) was harvested on October 31 and

Nov 01, 2013. All cattle were shipped 115 km to Creekstone Farms, Arkansas City, KS for slaughter. The CONV and CONV-Z cattle were harvested on the respective Thursday, and the NAT cattle were harvested on the Friday of each harvest week. This difference in ship date was due to the requirements of the packing facility in that they only harvest NAT cattle on Friday's of each week. At the packing facility, liver and lungs from every steer were scored for abscesses and abnormalities, respectively. Hearts and liver samples were collected at the abattoir for histological analyses from the same subset of steers that was identified on d 0 of the study (6 per pen).

### ***Data Collection***

***Hematology.*** Blood samples (3 mL; K<sub>2</sub> EDTA, Becton Dickinson Vacutainer Systems) were collected via jugular venipuncture with an 18-gauge  $\times$  2.54 cm needle on d 0, 28, 56, 84, 0Z, 10Z and 20Z. Samples were immediately placed on ice and transported to the Oklahoma State University Animal Science Building. Samples were analyzed for total and differential white blood cell (WBC) determination, total red blood cells, total platelets, hemoglobin, and hematocrit (ProCyt Dx Hematology Analyzer, IDEXX Laboratories, Westbrook, ME).

***Blood pH, Glucose and Lactate.*** Blood samples (3 mL; Lithium Heparin, Becton Dickinson Vacutainer Systems) were collected via jugular venipuncture with an 18-gauge  $\times$  2.54 cm needle on d 0Z, 10Z and 20Z. These samples were analyzed on site using a blood gas analyzer (GEM Premiere 3000, Instrumentation Laboratory, Lexington, MA). Standards utilized for this analyzer were 39.1°C for subject temperature and 21% for environmental oxygen concentration. Response variables included pH, sodium, potassium, calcium, glucose and lactate.

***C-reactive protein and BUN.*** Whole blood samples (10 mL; Becton Dickinson Vacutainer System) were collected into vacutainer tubes containing no additives on d 0, 56, 0Z, 10Z and 20Z. Whole blood was allowed to clot for 24 h at 4°C and serum was collected after centrifugation at 2,500  $\times$  g for 20 min at 4°C. Serum was harvested in 2-mL centrifuge tubes and stored at -20°C until

further analyses were performed (BioLis 24i Chemistry Analyzer, Carolina Liquid Chemistries Corporation, Winston-Salem, NC).

***Lung, Heart and Liver Scoring and Analysis.*** At the abattoir lungs from every steer were palpated for abnormalities, unless the visceral was condemned by the USDA inspector. Bronchopneumonia and pleural adhesion scores were assigned to lobes on the left and right side individually, while interlobular adhesions and missing tissue scores were assigned to the lungs as a whole. The lung scoring system used was adapted from Thompson et al., (2006) and Bryant et al., (1999). Bronchopneumonia scores were: 0 = no visible or palpable lesions or mild hyperemia of the cranioventral lung lobes without any consolidation; 1 = consolidation of up to 25% of the cranioventral lobe(s); 2 = consolidation of 26-50% of the cranioventral lobe(s); 3 = consolidation of greater than 50% of the cranioventral lobe(s). Pleural adhesion scores were: 0 = no adhesions or pleuritis or missing tissue; 1 = up to 25% adhesions or pleuritis; 2 = 26-50% adhesions or pleuritis; 3 = greater than 50% adhesions or pleuritis. Interlobular adhesion scores were: 0 = no adhesions; 1 = adhesions present. Missing tissue scores were: 0 = no missing tissue; 1 = missing tissue detected. Lungs from 23 steers were unaccounted for due to collection error on the first harvest day. These all originated from one weight block, and as a result that weight block was excluded from the lung score data analyses (n = 7). Liver scores were also obtained on every steer by recording the size and number of abscesses present. Liver scores O, A, and A+ were utilized as described by Brown and Lawrence (2010). Whole hearts and liver samples were collected from the previously described subset of steers. These tissues were placed on ice and transported to Oklahoma State University Laboratory. Upon arrival (~3 h post-harvest), individual hearts were weighed and three sections of the heart (left ventricular free wall, right ventricular free wall and interventricular septum adjacent to a papillary muscle) and one random section of the liver were immersed in 10% buffered formalin. These samples were sent to the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL, Amarillo, TX) for histological examination by blinded pathologists. Histological diagnosis for the

liver samples included hepatitis, hepatic telangiectasia and hepatic portal fibrosis, and bile duct hyperplasia. Histological diagnosis for the heart samples included myocarditis and cardiomyopathy. Any histological change was categorized as “abnormal” for analysis.

### ***Statistical Analysis***

All data were analyzed from a randomized complete block design, with pen considered the experimental unit and weight block included as a random effect. The percentage of cattle with liver abscesses and percentage of “abnormal” histology diagnoses were analyzed utilizing PROC GLIMMIX (SAS 9.3; SAS Inst. Cary, NC). Mixed models repeated measures methods were used, and fit statistics were compared to determine covariance structure for variables measured over time. Differences were considered significantly different when  $P \leq 0.05$ , and a trend when  $0.05 < P \leq 0.10$ .

## **RESULTS**

For detailed feedlot performance and carcass characteristic results please refer to Maxwell et al., (2014b). Briefly, the CONV cattle had a 32% increase in ADG and 26% improvement in efficiency versus the NAT cattle ( $P < 0.01$ ; data not shown). The CONV-Z cattle had a 34% increase in ADG and 33% improvement in efficiency versus the NAT cattle ( $P < 0.01$ ; data not shown). Three steers died during the study (1-NAT; 1-CONV-Z prior to the ZH feeding period; 1-CONV-Z during the ZH feeding period) with gross necropsies indicating bloat as the cause of death. No steers required treatment for respiratory disease during this study. Although statistical differences were detected for certain blood parameters, these resulting values were still within clinically normal concentrations throughout the experiment (Table 4.1).

Hematology results are represented in Table 4.2 and 4.3. Treatment did not influence the count of red blood cells, reticulocytes, hemoglobin or hematocrit ( $P \geq 0.35$ ). A treatment  $\times$  time interaction was noted for total white blood cells, with the CONV and CONV-Z steers having

significantly greater counts than NAT steers from d 28 (9.83 and 9.54 vs.  $8.61 \times 10^6/\mu\text{L}$ , respectively;  $P = 0.03$ ) through d 10Z (11.27 and 11.52 vs  $9.96 \times 10^6/\mu\text{L}$ , respectively;  $P < 0.01$ ) and a tendency on d 20Z (10.83 and 11.25 vs.  $9.83 \times 10^6/\mu\text{L}$ , respectively;  $P = 0.07$ ). This change was primarily driven by neutrophil counts which were greater for CONV and CONV-Z versus NAT steers from d 28 (2.57 and 2.47 vs.  $1.99 \times 10^3/\mu\text{L}$ , respectively;  $P < 0.04$ ) through d 20Z (3.51 and 3.47 vs.  $2.44 \times 10^3/\mu\text{L}$ , respectively;  $P < 0.01$ ). Lymphocyte counts were not affected by treatment ( $P = 0.43$ ), but the neutrophil-to-lymphocyte ratio was greater for CONV and CONV-Z steers compared to NAT steers (0.54 and 0.52 vs. 0.40, respectively;  $P < 0.01$ ). Overall, CONV and CONV-Z steers displayed a greater number of monocytes than NAT steers (1.21 and 1.22 vs.  $1.08 \times 10^3/\mu\text{L}$ , respectively;  $P < 0.01$ ). Treatment did not affect platelet counts ( $P = 0.99$ ).

Blood pH was greater for CONV-Z than NAT steers on d 10Z (7.41 vs. 7.37;  $P = 0.02$ ), and intermediary for CONV steers (7.39; Table 4.4). At d 20Z, CONV-Z steers had greater pH levels than CONV and NAT steers (7.39 vs. 7.37 and 7.38, respectively;  $P < 0.05$ ). Calcium and sodium concentrations were not affected by treatment ( $P \geq 0.70$ ), but potassium concentration were elevated at d 10Z for the CONV-Z steers compared to the NAT and CONV steers (4.40 vs. 4.17 and 4.23  $\text{mmol}\cdot\text{dL}^{-1}$ , respectively;  $P \leq 0.01$ ). By d 20Z the CONV-Z cattle had concentrations greater than the NAT steers (4.30 vs 4.15  $\text{mmol}\cdot\text{dL}^{-1}$ ;  $P = 0.01$ ) and CONV were intermediary (4.23). During the final 20 d on feed, the CONV steers had greater circulating concentration of glucose than NAT and CONV-Z steers (88.9 vs. 83.9 and 82.5  $\text{mg}\cdot\text{dL}^{-1}$ , respectively;  $P = 0.01$ ). Zilpaterol hydrochloride caused a reduction in circulating lactate concentration compared to CONV and NAT steers at d 10Z (13.5 vs 27.3 and 28.9  $\text{mg}\cdot\text{dL}^{-1}$ , respectively;  $P < 0.01$ ) and d 20Z (12.5 vs 27.2 and 25.1  $\text{mg}\cdot\text{dL}^{-1}$ , respectively;  $P < 0.01$ ).

Blood urea nitrogen (BUN) and C-reactive protein (CRP) results displayed a treatment  $\times$  time interaction and are presented in Table 4.5. Blood urea nitrogen concentrations were reduced on d 0Z for both groups of conventional steers compared to NAT steers (16.2 vs 17.5;  $P < 0.02$ ).

Supplementing ZH decreased circulating urea nitrogen compared to NAT and CONV steers by d 10Z (14.7 vs. 18.0 and 18.6, respectively;  $P < 0.01$ ) and d 20Z (14.6 vs. 16.3 and 16.9, respectively;  $P \leq 0.02$ ). Serum analysis resulted in greater CRP concentrations for CONV-Z and CONV steers compared to NAT steers on d 10Z (7.21 and 7.55 vs. 6.07, respectively;  $P < 0.01$ ) and d 20Z (7.51 and 7.40 vs. 5.97, respectively;  $P < 0.01$ ).

All lung palpation results were averaged and summarized by pen (Table 4.6). Treatment had no effect on the distribution of lung scores ( $P \geq 0.11$ ; data not shown). The mean score for pleural adhesion on the left side tended to be greater for CONV-Z steers than NAT steers (0.57 vs. 0.29;  $P = 0.09$ ) and intermediary for CONV steers (0.48). Palpation for other lung abnormalities resulted in no effect of treatment ( $P \geq 0.21$ ). The percentage of abscessed livers and the histology results of liver abnormalities were not affected by treatment ( $P \geq 0.62$ ; Table 4.7). Absolute heart weights were heavier for CONV and CONV-Z steers than NAT steers (2219 and 2176 vs. 1983 g, respectively;  $P < 0.01$ ), but treatment had no effect when heart weights were expressed in relation to shrunk final BW ( $P = 0.46$ ). There was no effect of treatment on the percentage of abnormal hearts ( $P = 0.45$ ).

## DISCUSSION

Due to the low number of mortalities (three), this data could not be statistically analyzed and no cattle were treated for clinical respiratory disease. Utilizing 1600 steers and the same implant as the current study, Munson et al. (2012) reported no differences in morbidity (26.6%) or mortality (8.5%) when high risk calves were implanted on arrival or delayed 45 d before implantation. Utilizing seven different sequences of growth promoting implants (one control and six treatments), Smith et al. (1999) concluded that frequency of medical treatment and death loss (2.5%) did not differ across treatments for Holstein calves. Increasing the concentration of zeranol when implanting sheep caused a linear increase in mortality (Eckerman et al., 2013) in one study, but no differences were

reported in another study (Salisbury et al., 2007). Loneragan et al. (2014) concluded that mortality in feedlot cattle is rare (less than 0.5%, the final 24-29 d on feed), but administering BAA does increase the risk of death. These conclusions were drawn from large data sets (totaling more than 950,000 head), but it is important to note that these were retrospective analysis of mostly observational and unequally represented data sets. One data set was from controlled, randomized studies, but even those were not designed to investigate mortality. The authors also mention that there may be an unknown, confounding reason why a portion the cattle from the observational data sets were not fed a BAA. Perhaps they were targeting a value-added program or the cattle were projected to be too heavy if fed a BAA. Finally, the authors hypothesize that perhaps it is the change in management and feeding that is required during the BAA period that negatively affects the cattle and not an affect directly related to the feed additives. Large-scale, controlled and randomized experiments need to be conducted to fully investigate these potential concerns.

Mean corpuscular volume was greater for both conventional treatments compared to NAT steers, but no other red blood cell parameter measured was affected by treatment. Results for reticulocytes, hemoglobin and mean corpuscular hemoglobin concentrations were greater than “normal” concentrations for all three treatments. Clenbuterol administration for 28 d had no effect on hemoglobin concentration or percentage of hematocrit in calves (Bruckmaier and Blum, 1992). Supplementing finishing steers and heifers with ZH did not affect hematocrit percentage (Burson et al., 2014). Implantation with 36 mg of zeranol did not alter hematocrit percentages in feeder calves (Phillips et al., 1986). Smith et al. (1999) and Richeson et al. (2013) reported that various implant strategies had no effect on red blood cells, hemoglobin or hematocrits of Holstein calves or stocker beef calves, respectively. Mean corpuscular volume was not specifically discussed in these previous studies, but values for all treatments in the current study were within clinically normal ranges.

These technologies increased total white cell counts starting on d 28 and continued through d 20Z in the current study. This result was primarily driven by an increase in neutrophils during this

same period. Both CONV and CONV-Z steers had a greater neutrophil-to-lymphocyte ratio and an increased count of monocytes compared to NAT steers, but no differences between the two conventional groups. Li et al. (2000) concluded that BAA (L-646,969) alone had no effect on leukocyte profile or lymphocyte function when supplemented to finishing lambs for four weeks. Implanting stocker calves with 200 mg of progesterone and 20 mg of estradiol benzoate had no effect on total or differentiated white blood cell counts (Richeson et al., 2013). Utilizing that same implant in Holstein steers increased the total white cell counts versus non-implanted calves, but did not affect the profile of white blood cells (Smith et al., 1999). Since no steers displayed clinical signs indicative of bovine respiratory disease during this finishing study, it was assumed that these steers did not experience an infectious disease stressor. This finishing trial took place during the summer of 2013 in which the comprehensive climate index frequently exceeded 35 (threshold for “severe” environmental stress; Mader et al., 2010) and visual signs indicated these cattle were experiencing heat stress (heat stress details are published elsewhere; Bernhard et al., 2014). A recent publication by Gifford et al. (2014) concluded that utilizing combination (TBA/estradiol) growth promoting implants reduces serum cortisol production. Yeager et al. (2011) proposed a biphasic mechanism of cortisol effect on immune function where varying concentrations of cortisol can be either pro- or anti-inflammatory. High levels of glucocorticoids can suppress the inflammatory response, while moderate levels can increase receptors for pro-inflammatory cytokines, extend neutrophil lifespan, and activate macrophages. Cortisol was not measured in the current study, but perhaps the combination implant was moderating cortisol production, which could support an immune response and help explain the moderate increases in immune cells. This could also help explain variations compared to previously cited literature in which combination implants were not utilized. Further research is needed to investigate the use of these technologies on hematology of finishing cattle.

Burson et al. (2014) reported no effects of ZH on blood pH. While in the current study, pH was greater for CONV-Z steers compared to NAT steers at d 10Z and greater for CONV-Z steers than

NAT and CONV steers by d 20Z. Sodium and calcium were not affected by treatment, but calcium was greater than “normal” for all three treatments. Potassium concentrations were greatest for CONV-Z cattle on d 10Z and 20Z. Burson et al. (2014) agreed that ZH supplementation increased potassium supplementation, but Burson also concluded that ZH supplementation reduced calcium concentration. Burson’s results would indicate that ZH caused a biologically significant difference in the cation-anion difference. In regard to implantation, serum potassium concentrations were not affected by treatment when Angus feeder calves were administered 36 mg of zeranol (Phillips et al., 1985). In the current study, supplementing ZH resulted in a decrease in lactate concentrations (50%), while glucose concentrations were significantly decreased in NAT and CONV-Z steers compared with CONV steers. This major drop in circulating lactate may help explain the increase in pH (previously discussed) when ZH is supplemented. These glucose and lactate results are supported by Bruckmaier and Blum (1992) who supplemented clenbuterol to calves for 35 days and concluded no differences in glucose concentration and a 33% decrease in lactate concentration. Hansen et al. (1997) saw no effect of salbutamol supplementation on circulating glucose concentrations of pigs. Enright et al. (1993) injected finishing beef heifers daily with growth hormone-releasing factor and/or thyrotropin-releasing hormone in an attempt to achieve a similar response as when utilizing a growth promoting implant. That study contradicted our result in that growth hormones had no effect on glucose concentration. In feeder calves (Phillips et al., 1985) and finishing lambs, (Wiggins et al., 1976; Wilson et al., 1972) zeranol implantation did not affect glucose concentrations either. All three of the previously mentioned studies concluded that treatment caused none to minimal (0-5.6%) improvements in performance, compared to the current study in which ADG was improved 32% in the CONV versus NAT steers. The implant utilized in the current study would be considerably more aggressive than zeranol implants or growth hormone injections. These differing results could also be partially contributed to the variation in mode of actions for zeranol implants and injected growth hormones versus combination (TBA/estradiol) implants. Zeranol is a synthetic “estrogen-like”

compound that can alter metabolism directly through cellular receptors or indirectly through increase production of growth hormones. Estradiol and TBA combination implants do not alter circulating growth hormone concentration, but still increase insulin-like growth factor-1 in circulation and at the tissue level, suggesting variation in mode of action when TBA is included.

At d 0Z blood urea nitrogen was lower for both conventional treatment groups, but a drastic reduction was concluded on d 10Z and 20Z as a result of ZH supplementation. In response to implantation, other scientist have reported mixed results depending on the implant protocol, but aggressive combination implants have typically reduced circulating urea nitrogen in finishing heifers and bulls (Bryant et al., 2010; Mader and Kreikemeier, 2006; Istasse et al., 1988). Supplementing BAA have been extremely consistent in decreasing blood urea nitrogen concentration in beef calves and sheep (Bruckmaier and Blum, 1992; Lopez-Carlos et al., 2012). The fact that implants and BAA decrease circulating urea nitrogen concentrations can be explained by the mode of action of these products, which promote muscle protein synthesis and utilization of nitrogen (Johnson and Chung, 2007)

Since no cattle exhibited clinical signs indicative of bovine respiratory disease during the finishing trial, it was surprising to have greater than 60% of the steers with evidence of consolidation on the right and/or left lobes and greater than 25% of the steers with evidence of adhesions on the right and/or left lobes. Munson et al. (2012) reported that 20% of “high risk” calves had greater than 5% pleural adhesion at harvest, irrespective of implantation time. Marchant-Forde et al. (2012) concluded that salbutamol had no effect on the percentage of pneumonia in palpated pig lungs. Since no steers in the current study displayed clinical signs indicative of respiratory disease during this trial, the investigators assume that this extensive amount of lung damage must have taken place during a previous production phase. Not knowing the health history of these steers, the investigators can only speculate that during the previous production phase, the steers either did not display clinical signs of illness or their signs were undiagnosed and untreated.

Coopridge et al. (2011) and the current study reported comparable percentages of abscessed livers and concluded no differences between natural and conventional treatments. Maxwell et al. (2014a) described a similar percentage (11%) of abscessed livers in the conventional cattle, but natural fed steers had nearly 40% condemned livers. The steers in Maxwell et al. (2014a) were fed a similar diet as the current study, but those steers had greater feed intakes and originated from a different genetic source. Using tylosin phosphate in feedlot rations has been reported to decrease the instance of liver abscesses by 40 to 70% (Nagaraja and Chengappa, 1998). The percentage of abnormal livers based on histology results was similar to the percentage of total abscessed livers and treatment had no effect on histology results. Burson et al., (2014) concluded that ZH supplementation did not affect liver histology results.

The BAA clenbuterol and cimaterol have been reported to induce hypertrophy of cardiac muscle and thus increase heart weight in rats and mice (Petrone et al., 1995; Eisen et al., 1988). Alternatively, salbutamol did not affect absolute heart weight or heart weight in relation to BW in pigs (Marchant-Forde et al., 2012; Hansen et al., 1997) or lambs (Sota et al. 1995). In the current study, BAA inclusion did not alter heart weight compared to the CONV steers, but both groups of conventional steers did have an increased absolute heart weight compared to NAT steers. This increase in heart weight was proportional to the increase in BW for these steers, leading the authors to believe that hypertrophic muscle accretion caused by conventional programs not only increased skeletal muscle, but proportionally alters cardiac muscle anabolism. Klindt et al. (1992; 1995) concluded that administration of increasing concentrations of porcine somatotropin linearly increased heart weights in finishing pigs. Alternatively, implanting sheep with TBA/estradiol benzoate did not alter heart weights in lambs fed concentrate or forage diets, but these lambs were only implanted 32 d prior to harvest which may have limited the expected implant response (McClure et al., 2000). Burson et al., (2014) agreed with the current study that treatment did not alter the histologic results of heart tissue.

## **CONCLUSIONS**

Anabolic growth implants and beta-adrenergic agonist have consistently proven their value for increasing production efficiency, which improves resource utilization and reduces the overall environmental impact. It is also believed that these products increase efficiency not by improving health or preventing disease, but by increasing protein retention. As a result, some scientists have questioned if there are any negative “side-effects” when administering these FDA approved products to finishing cattle. This question has been minimally researched in feedlot cattle and further investigations are needed to confirm our results. In the current study, utilizing growth promoting technologies that are commonly accepted in current conventional feeding programs did not have a negative effect on the health parameters or overall well-being of finishing beef steers.

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Table 4.1. The clinically normal ranges for blood parameters in Bovine of all life stages.<sup>1</sup>

Item	Low	High
Hematology <sup>1</sup>		
Red Blood Cells, 10 <sup>6</sup> /μL	4.47	9.35
Reticulocyte, 10 <sup>3</sup> /μL	0.0	3.9
Hemoglobin, g·dL <sup>-1</sup>	7.4	12.8
Hematocrit, %	22.5	39.9
Mean Corpuscular Volume, fL	40.4	56.4
Mean Cell Hemoglobin Concentration, g·dL <sup>-1</sup>	30.2	33.5
Total White Blood Cells, 10 <sup>3</sup> /μL	2.71	17.76
Neutrophils, 10 <sup>3</sup> /μL	0.68	6.94
Lymphocytes, 10 <sup>3</sup> /μL	1.20	10.62
Monocytes, 10 <sup>3</sup> /μL	0.02	2.17
Platelets, 10 <sup>3</sup> /μL	147	663
Blood pH and Metabolites <sup>2</sup>		
pH	7.36	7.46
Sodium, mmol·L <sup>-1</sup>	135	144
Potassium, mmol·L <sup>-1</sup>	3.6	5.0
Calcium, mmol·L <sup>-1</sup>	2.3	2.7
Glucose, mg·dL <sup>-1</sup>	53	76
Lactate, mg·dL <sup>-1</sup>	-	-
Blood Urea Nitrogen, mg·dL <sup>-1</sup>	6	18
C-reactive protein, mg·dL <sup>-1</sup>	-	-

<sup>1</sup>Reference ranges were obtained from: IDEXX ProCyte Dx Hematology Analyzer Reference Intervals, IDEXX Laboratories, Westbrook, ME

<sup>2</sup>Reference ranges were obtained: The College of Veterinarian Medicine Clinical Pathology Reference Intervals, Cornell University, Ithaca, NY

Table 4.2. The effects of treatment on red blood cell parameter data of finishing steers.

Item,	Treatment <sup>1</sup>			SEM <sup>2</sup>	P - value		
	NAT	CONV	CONV-Z		Trt	Time	Trt × Time
Red blood cells, 10 <sup>6</sup> /μL	8.71	8.54	8.54	0.10	0.35	<0.01	0.28
d 0	9.20	9.05	9.16				
d 28	8.49	8.14	8.24				
d 56	8.42	8.21	8.20				
d 84	8.69	8.52	8.51				
d 0Z	8.64	8.58	8.55				
d 10Z	8.73	8.55	8.55				
d 20Z	8.83	8.74	8.57				
Reticulocyte, 10 <sup>3</sup> /μL	5.49	5.84	5.64	0.23	0.58	<0.01	0.83
d 0	4.26	4.32	4.73				
d 28	6.60	6.45	5.81				
d 56	3.29	3.43	3.06				
d 84	6.09	6.37	6.58				
d 0Z	6.01	6.40	6.10				
d 10Z	5.81	6.66	6.53				
d 20Z	6.40	7.24	6.70				
Hemoglobin, g·dL <sup>-1</sup>	13.11	13.23	13.15	0.15	0.82	<0.01	0.40
d 0	12.84	12.77	12.96				
d 28	12.61	12.49	12.54				
d 56	12.95	13.19	13.04				
d 84	13.41	13.56	13.41				
d 0Z	13.27	13.56	13.43				
d 10Z	13.32	13.41	13.36				
d 20Z	13.38	13.65	13.34				
Hematocrit, %	38.6	39.4	39.1	0.6	0.50	<0.01	0.39
d 0	37.2	37.3	37.8				
d 28	37.0	37.1	37.1				
d 56	39.6	39.6	39.1				
d 84	39.8	40.9	40.5				
d 0Z	39.2	40.6	40.4				
d 10Z	39.1	39.8	39.6				
d 20Z	39.4	40.5	39.5				
Mean cell volume, fL	44.5 <sup>a</sup>	46.2 <sup>b</sup>	46.1 <sup>b</sup>	0.4	0.02	<0.01	0.49
d 0	40.4	41.3	41.4				
d 28	43.7	45.7	45.3				
d 56	46.1	48.3	47.9				
d 84	46.0	48.0	47.7				
d 0Z	45.6	47.4	47.4				
d 10Z	45.0	46.6	46.5				
d 20Z	44.8	46.4	46.3				
MCHC <sup>3</sup> , g·dL <sup>-1</sup>	34.0	33.6	33.7	0.2	0.22	<0.01	0.18
d 0	34.5	34.3	34.4				
d 28	34.2	33.7	33.8				
d 56	33.6	33.3	33.4				
d 84	33.7	33.2	33.2				
d 0Z	33.9	33.4	33.3				
d 10Z	34.1	33.7	33.8				
d 20Z	34.0	33.7	33.8				

<sup>a,b</sup>Means within a row without a common superscript differ ( $P \leq 0.05$ ).<sup>1</sup>Treatments include 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z).<sup>2</sup>Standard error of the mean (n = 8).<sup>3</sup>MCHC = Mean Cell Hemoglobin Concentration.

Table 4.3. The effects of treatment on white blood cell parameter data of finishing steers.

Item,	Treatment <sup>1</sup>			SEM <sup>2</sup>	P - value		
	NAT	CONV	CONV-Z		Trt	Time	Trt × Time
Total white cells, 10 <sup>3</sup> /μL	9.22	10.14	10.33	0.21	<0.01	<0.01	<0.01
d 0	7.98	8.24	8.33				
d 28	8.61 <sup>a</sup>	9.83 <sup>b</sup>	9.54 <sup>b</sup>				
d 56	8.24 <sup>a</sup>	9.02 <sup>b</sup>	9.46 <sup>b</sup>				
d 84	9.09 <sup>a</sup>	10.45 <sup>b</sup>	10.42 <sup>b</sup>				
d 0Z	10.83 <sup>a</sup>	11.33 <sup>ab</sup>	11.79 <sup>b</sup>				
d 10Z	9.96 <sup>a</sup>	11.27 <sup>b</sup>	11.52 <sup>b</sup>				
d 20Z	9.83 <sup>y</sup>	10.83 <sup>z</sup>	11.25 <sup>z</sup>				
Neutrophils, 10 <sup>3</sup> /μL	2.22	3.00	3.98	0.08	<0.01	<0.01	0.07
d 0	1.96	2.07	2.06				
d 28	1.99 <sup>a</sup>	2.57 <sup>b</sup>	2.47 <sup>b</sup>				
d 56	2.03 <sup>a</sup>	2.73 <sup>b</sup>	2.94 <sup>b</sup>				
d 84	2.18 <sup>a</sup>	3.36 <sup>b</sup>	3.17 <sup>b</sup>				
d 0Z	2.59 <sup>a</sup>	3.28 <sup>b</sup>	3.21 <sup>b</sup>				
d 10Z	2.40 <sup>a</sup>	3.46 <sup>b</sup>	3.42 <sup>b</sup>				
d 20Z	2.44 <sup>a</sup>	3.51 <sup>b</sup>	3.47 <sup>b</sup>				
Lymphocytes, 10 <sup>3</sup> /μL	5.65	5.63	5.82	0.19	0.43	<0.01	0.94
d 0	4.88	4.99	5.11				
d 28	5.33	5.63	5.60				
d 56	4.96	4.94	5.08				
d 84	5.45	5.46	5.52				
d 0Z	6.52	6.27	6.71				
d 10Z	6.26	6.22	6.43				
d 20Z	6.16	5.93	6.30				
N:L <sup>3</sup>	0.40 <sup>a</sup>	0.54 <sup>b</sup>	0.52 <sup>b</sup>	0.02	<0.01	<0.01	0.18
d 0	0.41	0.43	0.42				
d 28	0.37	0.46	0.44				
d 56	0.41	0.56	0.59				
d 84	0.41	0.62	0.60				
d 0Z	0.40	0.52	0.49				
d 10Z	0.38	0.56	0.54				
d 20Z	0.40	0.59	0.56				
Monocytes, 10 <sup>3</sup> /μL	1.08 <sup>a</sup>	1.21 <sup>b</sup>	1.22 <sup>b</sup>	0.03	<0.01	<0.01	0.33
d 0	1.03	1.10	1.06				
d 28	1.16	1.48	1.32				
d 56	1.03	1.06	1.15				
d 84	1.08	1.24	1.24				
d 0Z	1.23	1.30	1.45				
d 10Z	1.06	1.23	1.27				
d 20Z	0.95	1.07	1.05				
Platelets, 10 <sup>3</sup> /μL	463	462	465	14	0.99	<0.01	0.54
d 0	510	468	498				
d 28	511	487	514				
d 56	438	462	455				
d 84	438	467	453				
d 0Z	439	452	461				
d 10Z	448	430	419				
d 20Z	458	482	455				

<sup>a,b</sup>Means within a row without a common superscript differ ( $P \leq 0.05$ ).<sup>y,z</sup>Means without a common superscript differ ( $0.05 < P \leq 0.10$ ).<sup>1</sup>Treatments include 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z).<sup>2</sup>Standard error of the mean (n = 8).<sup>3</sup>Neutrophil-to-Lymphocyte ratio

Table 4.4. The effects of treatment on blood pH and metabolite concentrations of finishing steers.

Item,	Treatment <sup>1</sup>			SEM <sup>2</sup>	<i>P</i> - value		
	NAT	CONV	CONV-Z		Trt	Time	Trt × Time
pH	7.37	7.38	7.40	0.01	0.03	<0.01	0.04
d 0Z	7.37	7.38	7.38				
d 10Z	7.37 <sup>a</sup>	7.39 <sup>ab</sup>	7.41 <sup>b</sup>				
d 20Z	7.38 <sup>a</sup>	7.37 <sup>a</sup>	7.39 <sup>b</sup>				
Sodium, mmol·L <sup>-1</sup>	142.7	142.2	142.6	0.7	0.80	0.80	0.57
d 0Z	144.0	141.6	142.9				
d 10Z	142.1	142.7	142.4				
d 20Z	142.1	142.4	142.5				
Potassium, mmol·L <sup>-1</sup>	4.16	4.23	4.29	0.05	0.10	<0.01	<0.01
d 0Z	4.17	4.23	4.16				
d 10Z	4.17 <sup>a</sup>	4.23 <sup>a</sup>	4.40 <sup>b</sup>				
d 20Z	4.15 <sup>a</sup>	4.23 <sup>ab</sup>	4.30 <sup>b</sup>				
Calcium, mmol·L <sup>-1</sup>	4.82	4.85	4.83	0.02	0.70	<0.01	0.75
d 0Z	4.86	4.87	4.87				
d 10Z	4.76	4.79	4.80				
d 20Z	4.84	4.88	4.83				
Glucose, mg·dL <sup>-1</sup>	83.9 <sup>a</sup>	88.9 <sup>b</sup>	82.5 <sup>a</sup>	2.1	0.01	<0.01	0.38
d 0Z	86.7	91.0	87.9				
d 10Z	81.3	87.3	78.1				
d 20Z	83.7	88.2	81.8				
Lactate, mg·dL <sup>-1</sup>	29.1	28.8	18.7	2.4	<0.01	<0.01	0.01
d 0Z	33.3	31.9	30.1				
d 10Z	28.9 <sup>b</sup>	27.3 <sup>b</sup>	13.5 <sup>a</sup>				
d 20Z	25.1 <sup>b</sup>	27.2 <sup>b</sup>	12.5 <sup>a</sup>				

<sup>a,b</sup>Means within a row without a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>Treatments include 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z).

<sup>2</sup>Standard error of the mean (n = 8).

Table 4.5. The effects of treatment on blood urea nitrogen and C-reactive protein finishing steers.

Item,	Treatment <sup>1</sup>			SEM <sup>2</sup>	P - value		
	NAT	CONV	CONV-Z		Trt	Time	Trt × Time
BUN, mg·dL <sup>-1</sup>	15.8	15.5	14.4	0.3	<0.01	<0.01	<0.01
d 0	12.4	12.3	12.9				
d 56	15.1	13.6	13.4				
d 0Z	17.5 <sup>b</sup>	16.2 <sup>a</sup>	16.2 <sup>a</sup>				
d 10Z	18.0 <sup>b</sup>	18.6 <sup>b</sup>	14.7 <sup>a</sup>				
d 20Z	16.3 <sup>b</sup>	16.9 <sup>b</sup>	14.6 <sup>a</sup>				
C-reactive protein, mg·dL <sup>-1</sup>	5.54	6.26	5.97	0.25	0.07	<0.01	<0.01
d 0	4.10	4.06	3.69				
d 56	4.65	4.58	4.62				
d 0Z	6.91	7.69	6.82				
d 10Z	6.07 <sup>a</sup>	7.55 <sup>b</sup>	7.21 <sup>b</sup>				
d 20Z	5.97 <sup>a</sup>	7.40 <sup>b</sup>	7.51 <sup>b</sup>				

<sup>a,b</sup>Means within a row without a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>Treatments include 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z).

<sup>2</sup>Standard error of the mean (n = 8).

Table 4.6. The effects of treatment on palpated lung scores of finishing steers.

Item	Treatment <sup>1</sup>			SEM <sup>2</sup>	P-value <sup>2</sup>
	NAT	CONV	CONV-Z		
Right bronchopneumonia <sup>3</sup>	1.42	1.55	1.51	0.19	0.89
Left bronchopneumonia <sup>3</sup>	0.98	1.32	1.33	0.21	0.43
Right pleural adhesion <sup>4</sup>	0.57	0.71	0.90	0.12	0.21
Left pleural adhesion <sup>4</sup>	0.29	0.48	0.57	0.09	0.09
Interlobular adhesion <sup>5</sup>	0.39	0.42	0.49	0.05	0.42
Missing tissue <sup>6</sup>	0.16	0.23	0.20	0.06	0.65

<sup>1</sup>Treatments include 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z).

<sup>2</sup>Standard error of the mean (n = 7). P-value is for overall ANOVA.

<sup>3</sup>Mean bronchopneumonia score (0-3): 0 = no visible or palpable lesions or mild hyperemia of the cranioventral lung lobes without any consolidation; 1 = consolidation of up to 25% of the cranioventral lobe(s); 2 = consolidation of 26-50% of the cranioventral lobe(s); 3 = consolidation of greater than 50% of the cranioventral lobe(s).

<sup>4</sup>Mean pleural adhesion score (0-3): 0 = no adhesions or pleuritis or missing tissue; 1 = up to 25% adhesions or pleuritis; 2 = 26-50% adhesions or pleuritis; 3 = greater than 50% adhesions or pleuritis.

<sup>5</sup>Mean interlobular adhesion score (0-1): 0 = no adhesions; 1 = adhesions present.

<sup>6</sup>Mean missing tissue score (0-1): 0 = no missing tissue; 1 = missing tissue detected.

Table 4.7. The effects of treatment on liver and heart abnormalities of finishing steers.

Item	Treatment <sup>1</sup>			SEM <sup>2</sup>	P-value <sup>2</sup>
	NAT	CONV	CONV-Z		
Total liver abscesses, %	13.6	12.9	16.5	3.7	0.74
Liver abnormalities <sup>3</sup> , %	15.9	7.0	12.2	5.5	0.62
Heart weight, g	1983 <sup>a</sup>	2219 <sup>b</sup>	2176 <sup>b</sup>	31	<0.01
Heart weight/BW, g/kg	3.59	3.65	3.57	0.05	0.46
Heart weight/HCW, g/kg	5.71	5.76	5.54	0.08	0.11
Heart abnormalities <sup>4</sup> , %	14.9	9.4	11.5	6.9	0.45

<sup>a,b</sup>Means within a row without a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>Treatments include 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z).

<sup>2</sup>Standard error of the mean (n = 8). P-value is for overall ANOVA.

<sup>3</sup>Histologic changes included hepatitis, hepatic telangiectasia and hepatic portal fibrosis, and bile duct hyperplasia. Any histologic change was categorized as “abnormal”.

<sup>4</sup>Histologic changes included myocarditis and cardiomyopathy. Any histologic change was categorized as “abnormal”.

## CHAPTER V

### EFFECTS OF GROWTH-PROMOTING TECHNOLOGIES ON HEAT STRESS OF FINISHING STEERS

**ABSTRACT:** Crossbred steers ( $n = 252$ ; initial BW =  $401 \pm 8$  kg) were utilized in a randomized complete block design to determine the effects of technology use in feedlot production systems on heat stress. Treatments consisted of an all-natural treatment (defined as receiving no growth promoting technologies; **NAT**), a conventional treatment (implanted with 40 mg of estradiol and 200 mg of trenbolone acetate on d 0, and fed 33 and 9 mg/kg [DM basis] of monensin and tylosin daily, respectively; **CONV**) and a CONV treatment plus the addition of zilpaterol hydrochloride (**ZH**; at 8.33 mg/kg [90% DM basis] for the last 20 d on feed with a 3 to 4 d withdrawal; **CONV-Z**). Pen panting scores (**PS**) were assessed daily (starting on d 29) at 1700 h. Blood was collected every 10 d during the ZH feeding period (designated as d 0Z, 10Z and 20Z) on 6 steers/pen to determine blood gases. The same subset of steers was evaluated for core body temperature (**BT**), infrared thermography (**IT**) and hair covering score (**HS**) throughout the study. Individual PS and respiration rates (**RR**) were collected every other day during the final 23 DOF. Core BT was not significantly affected by treatment ( $P \geq 0.10$ ). A treatment  $\times$  time interaction ( $P \leq 0.03$ ) was detected for HS and IT. The degree of hair covering was less for CONV and CONV-Z steers versus NAT steers from d 84 (1.58 and 1.71 vs 2.01;  $P < 0.05$ ) through d 20Z (1.20 and 1.26 vs 1.98;  $P < 0.05$ ). Natural steers had cooler IT results from d 2Z ( $P = 0.04$ ) through d 22Z ( $P = 0.01$ ). During the ZH feeding period, CONV-Z cattle demonstrated increased severity in morning PS compared to CONV and NAT cattle (1.23 vs. 1.00 and 1.08, respectively;  $P < 0.03$ ), and the afternoon PS compared to CONV cattle (1.84

vs. 1.67;  $P < 0.01$ ), with NAT cattle intermediary (1.76). Respiration rates were lowest for CONV cattle, intermediate for NAT cattle, and highest for CONV-Z cattle in the morning (99.5 vs. 105.0 vs. 112.8 breaths/min, respectively;  $P < 0.01$ ) and afternoon (120.1 vs. 125.8 vs. 133.8 breaths/min, respectively;  $P < 0.01$ ). Blood measurements resulted in no effect of treatment on CO<sub>2</sub>, O<sub>2</sub> or oxygen saturation ( $P \geq 0.11$ ). Treatment altered the mechanism by which steers exchange heat load to maintain thermo homeostasis, but collectively, steers across all treatments experienced a similar magnitude of heat stress.

**Keywords:** beef cattle,  $\beta$ -adrenergic agonist, body temperature, conventional, heat stress, natural

## INTRODUCTION

The negative effects of heat stress has been estimated to cost the U.S. cattle industry \$2.4 billion dollars annually (St-Pierre et al., 2003). Recently, heat stress has been especially costly to feedlots in the central US where extreme heat waves have resulted in record mortalities “T. L. Mader (University of Nebraska, Concord, NE, personal communication).” Hahn and Becker (1984) describe heat stress as an event when total heat gain exceeds the animal’s heat loss capabilities, causing increased body temperature, disrupted behaviors, and impaired physiological function. Indicators of heat stress (i.e. respiration rate and body temperature) are typically greater in dark-hided cattle (Arp et al., 1983; Mader et al., 2006). In 1996, Busby and Loy concluded that greater than 75% of feedlot deaths caused by heat stress were dark-hided cattle. The risk of heat stress is also increased late in the feeding period, because the addition of external fat can alter heat exchange.

Implanting with exogenous growth promotants and supplementing beta-adrenergic agonist (BAA) will modify nutrient partitioning, increasing growth rate and protein anabolism. Increases in body mass in relation to surface area could lead to a greater risk of heat stress. Beta-adrenegic agonist cause arteriolar dilation, which has led to increased heart and respiration rates (RR;

Bruckmaier and Blum, 1992; Eiler, 2004). Macias-Cruz et al. (2010) concluded that feeding BAA increased skin temperature of lambs in a heat stress environment. Recently, anecdotal reports have suggested a potential link between an animal's response to heat stress (ultimately cattle mortality) and the feeding of BAA. The objective of this study was to determine the effects of growth-promoting technologies in conventional beef production systems with and without the use of a BAA on heat stress of finishing steers compared to an all-natural production system.

## **MATERIALS AND METHODS**

All protocols were approved by the Oklahoma State University Institutional Animal Care and Use Committee.

### ***Cattle Management***

A more detailed description of cattle management in this experiment is published elsewhere (Maxwell et al., 2014). In April of 2013, 423 black-hided certified natural steers were transported from Willow Lake, SD (n = 303), and Cedar Rapids, NE (n = 120) to the Willard Sparks Beef Research Center, Stillwater, OK. After processing, 171 steers were sorted off due to weight and used in another experiment. A total of 252 steers were enrolled in the present experiment on 2 different dates, May 07 and 09, 2013. Steers were weighed on d -1, blocked by BW within source and allocated to study pens. On d 0, all cattle were weighed, ruminal temperature recording boluses were administered orally and cattle sorted to study pens (6 blocks; 1 replication/block; 6 pens/treatment; 14 steers/pen; 84 steers/treatment; initial BW =  $401 \pm 8$  kg). Treatments consisted of an all-natural treatment (**NAT**), a conventional treatment (**CONV**), and a conventional treatment with the addition of a beta-agonist at the end of the feeding period (**CONV-Z**). The NAT cattle received no antibiotics, growth implants, or beta-agonists. The CONV and CONV-Z cattle were implanted with 40 mg of estradiol and 200 mg of trenbolone acetate (TBA; Revalor-XS<sup>®</sup>, Merck Animal Health) on d 0. They

were also fed 33 and 9 mg/kg (DM basis) of monensin and tylosin (Rumensin<sup>®</sup> and Tylan<sup>®</sup>, Elanco Animal Health, Greenfield, IN) daily, respectively. The CONV-Z cattle were fed zilpaterol hydrochloride (ZH; Zilmax<sup>®</sup>, Merck Animal Health) at 8.33 mg/kg (90% DM basis) for the last 20 d on feed, and ZH was withdrawn from feed for 3-4 d prior to slaughter. All cattle were fed the same base 93% concentrate diet as detailed by Maxwell et al. (2014). Briefly, the diet consisted of approximately 48% dry-rolled corn, 15% dried distiller grains, 15% wet corn gluten, 15% supplement (liquid and dry), and 7% switchgrass hay. All diets were formulated to meet or exceed NRC (2000) requirements.

Six steers per pen that represented the median BW of that pen were selected on d -1 as a pen subset. Ruminant blouses were placed on cattle in the subset groups on d 0 and blood samples were collected during the ZH feeding period. Cattle were weighed on d 0, 28, 56, and 84 of the finishing phase. On August 19 and 20, 2013, d 104 and 103, respectively, all cattle were weighed and the CONV-Z cattle were started on ZH. Measurements collected the morning prior to ZH supplementation is referenced to as d 0Z and spans through day 20Z, as ZH is typically fed for the last 20 days on feed. The cattle were also weighed on d 10Z and d 20Z. Cattle on CONV-Z were fed Zilmax at a rate of  $87.6 \text{ mg} \cdot \text{steer}^{-1} \cdot \text{d}^{-1}$ , which was based upon calculated intake and assayed zilpaterol values with a 3-4 d period of ZH withdrawal.

Cattle were fed for an average of 128 days. The cattle were harvest on September 12 and 13, 2013. All cattle were shipped 115 km to Creekstone Farms, Arkansas City, KS for harvest. The CONV and CONV-Z cattle were harvested on Thursday, and the NAT cattle were slaughtered on the Friday of the harvest week. This difference in ship date was due to the requirements of the packing facility in that they only harvest NAT cattle on Friday's of each week.

### ***Data Collection***

***Environmental Conditions.*** The comprehensive climate index (CCI) was utilized as the measure for environment heat load. This index provides an adjustment to ambient temperature for relative humidity, wind speed and solar radiation (Mader et al., 2010). The CCI can be utilized to predict cold or heat stress events in livestock. Table 5.1 represents the thermal stress thresholds during hot conditions that were previously published by Mader et al. (2010). Environmental data was collected at the National Weather Service's Stillwater, OK location, which is approximately 3.5 km northeast of the Willard Sparks Beef Research Center.

***Core Body Temperature.*** At the beginning of the study, six steers per pen were administered a remote temperature monitoring ruminal bolus (SmartStock, LLC., Pawnee, OK) using a custom designed balling gun. Boluses settled in the reticulum, and were programmed to record core body temperature (BT) once every 3 min and transmit individual animal data once every 15 min via fixed transceiver stations, which were specifically designed to receive bolus signals. A single transmission would include the previous 12 temperature readings to ensure maximum capture of temperature recordings. Transceiver stations were located in the fence line of every third pen and data were relayed to a fixed transceiver station equipped with a USB serial connection, which logged data in a database on a personal computer. On average 60% of every 3 min temperature reading was captured. From d 0 to d 0Z the data were summarized by pen per week, and data are presented per day during the ZH feeding period.

***Panting Score and Respiration Rate.*** Starting on d 29 panting scores (PS) were assigned to every animal in a pen every day at 1700 h. Scores were assigned by visual observations using a 0 to 4 scoring system adopted from Mader et al. (2006; Table 5.2). Every other day during the ZH feeding period, PS and respiration rates (RR) were assigned to the same subset of steers as previously described (6 steers/pen) at 1000 and 1700 h. Individual RR were measured by visual observation of flank movement. The time taken for 30 breaths was recorded using a stopwatch and utilized to calculate breaths·min<sup>-1</sup>.

***Hair Covering Score.*** Based on preliminary data collected at Willard Sparks Beef Research Center the previous summer, a hair covering scoring (HS) system was developed and utilized in this study. Scores (1-6) were assigned by a single trained technician on each weigh day (Table 5.3.)

***Infrared Thermography.*** Infrared body temperatures (IT) were collected in correspondence with each weigh period, but due to limited time and labor these data were collected 8 d after a respective weigh day during the majority of the study. Data were collected more frequent during the ZH period (d 2Z, d 12Z and d 22Z). Starting between 0400 and 0500 h the observer would capture a perpendicular video (from an approximate distance of 4-5 m) of the same subset of steers using an infrared thermography camera (Thermal CAM S65 HS, FLIR, Boston, MA). These videos were captured before sunrise to allow as much time as possible for night time cooling and to eliminate the effect of solar radiation. At a later date, the video was observed by a blinded technician who utilized Researcher Pro software (FLIR) to capture a still perpendicular image and determine the mean IT of each steer. To determine mean IT, a rectangular box was created that extended from the caudal border of the scapula to the cranial tuber coxae. Dorsally, the box extended approximately from the lateral vertebral spinous process to approximately level with the stifle. The software enabled the technician to determine a mean IT for the area previously described.

***Blood Gases.*** Blood samples (3 mL; Lithium Heparin, Becton Dickinson Vacutainer Systems) were collected via jugular venipuncture with an 18-gauge needle on d 0Z, 10Z and 20Z. These samples were immediately analyzed on site using a blood gas analyzer (GEM Premiere 3000, Instrumentation Laboratory, Lexington, MA). Response variables for blood gas analysis included partial O<sub>2</sub>, partial CO<sub>2</sub> and oxygen saturation.

### ***Statistical Analysis***

All data were analyzed from a randomized complete block design, with pen considered the experimental unit and weight block included as a random effect. Mixed models

repeated measures methods were used, and fit statistics were compared to determine covariance structure for variables measured over time (PROC GLIMMIX, SAS 9.3; SAS Inst. Cary, NC). Differences were considered significantly different when  $P \leq 0.05$ , and a trend when  $0.05 < P \leq 0.10$ .

## RESULTS

The steers utilized in this experiment were a portion of the experiment described by Maxwell et al. (2014). For detailed feedlot performance and carcass characteristic results please refer to Maxwell et al. (2014). Briefly, the CONV cattle had a 32% increase in ADG and 26% improvement in efficiency versus the NAT cattle ( $P < 0.01$ ; data not shown). The CONV-Z cattle had a 34% increase in ADG and 33% improvement in efficiency versus the NAT cattle ( $P < 0.01$ ; data not shown).

The minimum, average and maximum CCI are presented in Figure 5.1. These daily means were summarized and are presented on a weekly basis. Throughout the experiment the mean minimum, mean average and mean maximum CCI were 17.7, 25.9 and 36.9, respectively. Specifically during the ZH feeding period, the mean minimum, mean average and mean maximum CCI were 21.4, 29.8 and 40.9, respectively (Figure 5.2).

From d 0 to d 0Z, average daily body temperature was summarized by week and was not affected by treatment ( $P = 0.16$ ; Figure 5.1). A closer examination during the ZH feeding period revealed a tendency for a treatment  $\times$  time interaction ( $P = 0.10$ ; Figure 5.2), in which NAT steers were cooler than CONV steers on August 31<sup>st</sup> (40.4 vs. 40.8°C) and September 1<sup>st</sup> (40.1 vs. 40.5°C), 4<sup>th</sup> (39.7 vs. 40.0°C) and 11<sup>th</sup> (40.1 vs. 40.4°C;  $P \leq 0.05$ ) and NAT steers were cooler than CONV-Z steers on September 4<sup>th</sup> (39.7 vs. 40.0°C) and 11<sup>th</sup> (40.1 vs. 40.5°C;  $P < 0.05$ ). August 31<sup>st</sup> would represent d 12Z or 11Z relative to group 1 or 2, respectively.

Daily pen PS were also summarized on a weekly basis. Panting score prior to the ZH feeding period was not different between treatments ( $P = 0.23$ ; Figure 5.3). These PS were collected from

17:00 to 18:00 h and BT was averaged from 30 min prior to 30 min after the collection of PS. Weekly averages of these BT ranged from 40.3 to 41.3°C, with a mean of 40.7°C. Treatment had no effect on average BT prior to the ZH feeding period ( $P = 0.33$ ; Figure 5.3).

Individual PS and RR measured on a subset of each pen during the ZH feeding period were affected by treatment during the morning (Figure 5.4a) and evening (Figure 5.4b) collection periods ( $P < 0.01$ ). The CONV-Z steers had a greater panting score than CONV and NAT steers in the morning (1.23 vs. 1.00 and 1.08;  $P < 0.01$ ), while CONV-Z steers had a greater PS than CONV steers in the evening (1.84 vs. 1.67;  $P < 0.01$ ) and NAT steers were intermediary (1.76). Steers fed ZH exhibited the fastest RR, while NAT steers were intermediate and CONV steers were the slowest in the morning (112.8 vs. 105.0 vs. 99.5;  $P < 0.01$ ) and evening (133.8 vs. 125.8 vs. 120.1;  $P < 0.01$ ). Mean BT summarized from 30 min prior to 30 min after the collection of PS and RR resulted in no differences across treatment in the morning ( $P = 0.23$ ; Figure 5.5a) or evening ( $P = 0.14$ ; Figure 5.5b). Morning BT ranged from 39.1 to 39.9°C with a mean of 39.5°C, while evening BT ranged from 40.3 to 41.2°C with a mean of 40.9°C. Morning BT were cooler than evening BT for all treatments ( $P < 0.01$ ).

Hair covering scores were not different across treatments the first 56 d on feed, but CONV and CONV-Z steers shed their winter hair coats at a quicker rate resulting in a lower score from d 84 ( $P = 0.03$ ) to d 20Z ( $P < 0.01$ ; Table 5.4). Infrared temperatures yielded similar results with no differences across treatments through d 92, but CON and CONV-Z steers had greater IT on d 2Z ( $P = 0.04$ ) through d 22Z ( $P = 0.01$ ; Table 5.4).

Blood gas results are presented in table 5.5. No differences were detected for partial O<sub>2</sub>, partial CO<sub>2</sub> or oxygen saturation across treatments ( $P \geq 0.11$ ).

## DISCUSSION

In North Central Oklahoma, ambient temperatures typically peak in July through early August, but in 2013 the summer heat wave was shifted approximately 30 days with ambient temperatures peaking in August through early September (National Weather Service).

Based on the CCI data collected and the thermal stress thresholds published in Mader et al. (2010), these steers experienced severe to extreme heat stress during peak heating hours of the day during the majority on this experiment. Environmental conditions were particularly stressful during the ZH feeding period when minimum, average and maximum CCI values were approximately 4 units greater than prior to the ZH feeding period. Mader et al. (2010) explained that CCI reaching the “severe” threshold was capable of causing death of animals and the “extreme” threshold would have a high probability of causing death of high-risk animals. Heavy, finishing cattle have been previously considered to be at higher risk for heat stress due to the increasing mass to surface area ratio and the fact that subcutaneous fat can decrease heat exchange capabilities (Mader and Davis, 2004).

Rectal temperature is still considered the “gold standard” in regard to accessing BT in cattle. Manual collection of rectal temperatures is most common due to the ease of collection and low cost of rectal thermometers. Unfortunately, this process can be very labor intensive and time consuming, and can be altered by the competency of the operator and the amount of stress the animal experiences during the process. One alternative that has been investigated for its potential application in detecting illness, heat stress and estrus without human intervention is measuring temperature via remote monitoring devices. Recent advancements in technology have increased the reliability and decreased the cost of remote temperature monitoring. Ruminant temperature has been proven to be highly correlated ( $r = 0.65$  to  $0.92$ ) to rectal temperature and measured approximately  $0.5^{\circ}\text{C}$  warmer than rectal temperature in previous research (Sievers et al., 2004; Bewley et al., 2008; Tismsit et al., 2011; Rose-Dye et al., 2011; Wahrmond et al., 2012). As a result, this technology has become more popular in research settings and in dairy cattle production systems.

In this experiment, average daily core body temperatures ranged from 39.4 to 40.5°C, with a mean of 40.0°C over the 15 weeks prior to the ZH feeding period and was not affected by treatment. Mader and Kreikemeier (2006) concluded that growth promoting treatments (including multiple implant protocols) did not affect mean tympanic temperature of heifers during the summer or winter. Non-implanted heifers and heifers administered an estradiol-17 $\beta$  and a trenbolone acetate implant had an average tympanic temperature of 39.0°C. Based on a temperature and humidity index, the heifers in Mader and Kreikemeier (2006) were under heat stress during most of the summer sampling period. The lower average tympanic temperature (39.0°C) compared to the average ruminal bolus temperature (40.0°C) in the current study was not a surprise considering Prendiville et al. (2002) concluded that tympanic temperature measures 0.8°C cooler than ruminal temperatures in cattle. Wahrmond et al. (2014) supplemented ZH to conventionally fed steers and heifers during the fall, winter and spring, and reported that BT (measured by ruminal bolus) was not affected by treatment during the ZH feeding period. Body temperature averaged from 39.4 to 39.7°C for various groups of cattle. These temperature are lower than the 40.2°C average that was reported in the current study during the ZH period, but the cattle studied by Wahrmond et al. (2014) were not finished during the heat of the summer, which has proven to increase average rectal temperature by 0.7°C (Gaughan et al., 2005). The current study did detect a tendency for a treatment  $\times$  time interaction during the ZH feeding period for August 31<sup>st</sup>, September 1<sup>st</sup>, 4<sup>th</sup> and 11<sup>th</sup>. August 31, 2013 represented the hottest average daily BT (40.6°C) and hottest maximum CCI (46) of the entire study. A CCI of 46 falls within the “extreme danger” category of the thermal stress threshold. The days when treatment  $\times$  time differences in average BT were detected, the magnitude of differences was 0.3 to 0.4°C. The slight increases in ruminal temperature during the ZH feeding period were potentially due to the fact that the CONV and CONV-Z steers consumed 1.3 and 0.9 kg more DMI, respectively (data published elsewhere, Maxwell et al., 2014). Like average BT, feed intake across all treatments was similar until the last 23 d on feed. Reducing feed intake of a finishing ration to 75% ad libitum decreased tympanic

temperature greater than 0.5°C due to metabolic heat load (Mader et al., 2002). Variation in diet (low and moderate concentration versus high concentrate) have been shown to alter ruminal temperature by 0.2°C (Dye-Rose et al., 2009). These changes are contributed to alterations in heat of fermentation or metabolic heat load. An additional experiment was conducted simultaneously to the current study with a contemporary group of steers. In that study, natural steers consumed more water than conventional steers (Maxwell et al., 2014). This increase in average daily water intake could also help explain slight decreases in mean ruminal temperature in NAT steers. The measurements of the current study never detected a difference in average BT between CONV and CONV-Z steers, but Burson et al. (2014) revealed that rectal temperatures were greater in ZH supplemented steers. The author also stated that the difference of 0.02°C is likely not biologically significant.

During the ZH feeding period, average evening BT was 1.4°C higher than the average morning BT. During those same time points, CCI increased by 14 units (data not shown). Mader and Kreikemeier reported that tympanic temperature increased by 1.0°C from morning to evening during the summer. From 1600 to 1800 h, average BT was increased 0.9°C compared to average BT from 0900 to 1100 h (Eigenberg et al., 2005). Typically, BT will be higher in the evening versus the morning due to a building heat load resulting from an increasing ambient temperature and solar radiation. This increased ultimately creates a “normal” diurnal curve for cattle exposed to summer heat.

Utilizing a similar scoring system, Mader et al. (2008) reported mean PS of 1.0 and 1.6 in the morning and evening, respectively. These averages were slightly lower than the 1.1 (morning) and 1.8 (evening) reported in the current study. Mader et al. (2008) did agree with the results herein, that growth promoting implants did not have an effect on PS in cattle during hot environmental conditions. Gaughan et al. (2005) reported that growth implants had no effects on steer or heifer RR. In that study, RR averaged 114 breaths·min<sup>-1</sup>. This information contradicted the current study interpretation in which the NAT steers had increased RR (5.5 additional breaths·min<sup>-1</sup> compared to

CONV steers) to dissipate a greater heat load. The current study also demonstrated that ZH supplementation increased PS and RR ( $13.5 \text{ breaths} \cdot \text{min}^{-1}$  compared to CONV steers) the final 23 d on feed. Zilpaterol hydrochloride increases protein development and retention which should increase heat production. Overall, BT was not significantly increased by ZH supplementation, which suggested that these steers were able to offset the additional heat load through increasing RR. Burson et al. (2014) reported that PS was not affected by ZH supplementation (RR was not measured); but, it is important to note that those cattle were subject to less extreme heat condition than the current study. The increase in RR in CONV-Z steers in the current study is likely linked to the basic mode of action of BAA. Beta-agonist are known to be bronchodilators and vasodilators, causing an increase in RR and heart rate.

Hair growth and shedding are tightly controlled and regulated by complex mechanisms under hormonal control, but knowledge is lacking in regard to the mode of action and specific hormonal compounds responsible. Observations of this experiment strongly suggest that growth-promoting implants (estrogenic and androgenic compounds) promote the shedding of winter hair coats. The inability of NAT steers to fully shed their winter hair coat can have a significant impact on environmental heat exchange. Longer, rougher hair coats provide more insulation, resulting in less heat loss from the skin to the environment. These types of hair coats are also less resistant to heat transfer to the skin by solar radiation than smooth, shiny summer coats that reflect more radiation at or near the surface (Finch, 1986). Thermal imaging data from the current study support these statements, as NAT steers were not able to expel thermal heat through their body surface as effectively as CONV and CONV-Z steers. Macias-Cruz et al. (2010), determined that ZH supplementation to hair sheep during heat-stress conditions increased the belly and flank skin surface temperature by greater than  $4^{\circ}\text{C}$  ( $35.1$  vs.  $39.5^{\circ}\text{C}$ ). The authors suggested this may be partially due to ZH altering microbial fermentation, rumen digestion and intestinal environment. The skin temperatures reported by Macias-Cruz et al. (2010) are drastically greater than the thermal images

temperature collected in the current study. It is important to note that temperatures collected by Marcias-Cruz et al. (2010) were taken at 4 time points throughout the day meaning solar radiation would have played a major role in skin temperature, unlike the thermal images in the current study.

Burson et al. (2014), described that ZH supplemented cattle tended to have a lower partial pressure of oxygen, while partial pressure of carbon dioxide and oxygen saturation was not affected. These results vary slightly from the current study that detected no differences across treatments for these same three variables. The current study collected blood samples between 0400 and 0700 h, while Burson et al. (2014) collected blood samples during the day when the cattle may have been experiences more heat load due to the weighing and handling processes.

## **CONCLUSIONS**

Heat stress in feedlot cattle has been identified as economically important and is a growing animal welfare concern. Growth-promoting technologies have proven to be valuable tools for increasing beef production and efficiency, but recently, their effect on animal well-being during stressful environmental conditions has been questioned. The body of literature in this area (especially in regard to beta-agonists) needs to be further developed. In this experiment, the method by which these steers dealt with heat load was altered. Natural steers had a more difficult time transferring heat across the skin surface, so they potentially compensated by increasing respiration rate to improve evaporative heat exchange. Steers supplemented with ZH appeared to experience an increased heat load due to increased metabolic rate and increased body mass, but compensated through an increased respiration rate to maintain thermal neutrality. Collectively, the use of growth-promoting technologies did not affect the overall heat stress or animal welfare of finishing steers experiencing prolonged, hot environmental conditions, in the present experiment.

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Table 5.1. Arbitrary comprehensive climate index  
thermal stress threshold (Mader et al., 2010)

Environment	Hot Conditions
No Stress	<25
Mild	25 to 30
Moderate	>30 to 35
Severe	>35 to 40
Extreme	>40 to 45
Extreme Danger	>45

Table 5.2. Assessment used for panting scores (Mader et al., 2006)

Score	Description
0	Normal respiration
1	Elevated respiration
2	Moderate panting and/or presence of drool or small amount of saliva
3	Heavy open-mouthed panting; saliva usually present
4	Severe open-mouthed panting accompanied by protruding tongue and excessive salivation; usually with neck extended forward

Table 5.3. Assessment used for hair covering scores

Score	Description
1	Slick, shiny hair coat, no chance for mud attachment
2	Less than $\frac{1}{3}$ rough hair coat covering the body, some possibility of slight mud attachment
3	Between $\frac{1}{3}$ and $\frac{2}{3}$ rough hair coat covering the body, chance of moderate mud attachment
4	Greater than $\frac{2}{3}$ rough hair coat covering the body with initial signs of shedding (usually at the shoulder of hind quarter)
5	Full winter rough hair coat covering the body, possibility for extensive mud attachment
6	Full winter rough hair coat covering that is excessive in length, possible for extensive mud attachment

Table 5.4. The effects of treatment on hair covering score and infrared thermography of finishing steers.

Item,	Treatment <sup>1</sup>			SEM <sup>2</sup>	P - value		
	NAT	CONV	CONV-Z		Trt	Time	Trt × Time
Hair Covering Score <sup>3</sup>	2.77	2.34	2.39	0.19	< 0.01	< 0.01	0.03
d 0	4.82	4.81	4.78				
d 28	4.20	3.92	4.00				
d 56	2.11	1.99	2.08				
d 84	2.01 <sup>b</sup>	1.58 <sup>a</sup>	1.71 <sup>a</sup>				
d 0Z	2.20 <sup>b</sup>	1.46 <sup>a</sup>	1.55 <sup>a</sup>				
d 10Z	2.06 <sup>b</sup>	1.39 <sup>a</sup>	1.35 <sup>a</sup>				
d 20Z	1.98 <sup>b</sup>	1.20 <sup>a</sup>	1.26 <sup>a</sup>				
Infrared Temperament, °C <sup>4</sup>	30.0	30.6	30.7	0.13	<0.01	<0.01	0.01
d 36	30.0	30.2	30.2				
d 64	32.0	32.2	32.2				
d 92	30.0	30.5	30.5				
d 2Z	28.9 <sup>a</sup>	29.8 <sup>b</sup>	30.2 <sup>b</sup>				
d 12Z	30.1 <sup>a</sup>	31.0 <sup>b</sup>	31.4 <sup>b</sup>				
d 22Z	28.9 <sup>a</sup>	30.0 <sup>b</sup>	29.8 <sup>b</sup>				

<sup>a,b</sup>Means without a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>Treatments include 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z).

<sup>2</sup>Standard error of the mean (n = 6).

<sup>3</sup>Hide covering scores (1-6): 1 = slick, shiny hair coat, no chance for mud attachment; 2 = less than  $\frac{1}{3}$  rough hair coat covering the body, some possibility of slight mud attachment; 3 = between  $\frac{1}{3}$  and  $\frac{2}{3}$  rough hair coat covering the body, chance of moderate mud attachment; 4 = greater than  $\frac{2}{3}$  rough hair coat covering the body with initial signs of shedding (usually at the shoulder of hind quarter); 5 = full winter rough hair coat covering the body, possibility for extensive mud attachment; 6 = full winter rough hair coat covering that is excessive in length, possible for extensive mud attachment.

<sup>4</sup>Hide covering score was included in the model as a covariate.

Table 5.5. The effects of treatment on blood gases of finishing steers.

Item,	Treatment <sup>1</sup>			SEM <sup>2</sup>	P - value		
	NAT	CONV	CONV-Z		Trt	Time	Trt*Time
CO <sub>2</sub> , mmHG	50.3	49.0	48.9	1.1	0.58	<0.01	0.16
d 0Z	53.4	51.9	52.0				
d 10Z	49.2	45.9	46.0				
d 20Z	48.4	49.3	48.7				
O <sub>2</sub> , mmHG	72.7	74.2	66.9	3.1	0.11	0.35	0.89
d 0Z	72.2	75.8	68.1				
d 10Z	72.7	68.4	62.7				
d 20Z	73.1	78.3	69.9				
Oxygen Saturation, %	89.0	90.2	88.3	1.0	0.14	0.32	0.86
d 0Z	88.1	89.0	88.4				
d 10Z	88.9	90.3	87.3				
d 20Z	89.8	91.1	89.3				

<sup>1</sup>Treatments include 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z).

<sup>2</sup>Standard error of the mean (n = 6).

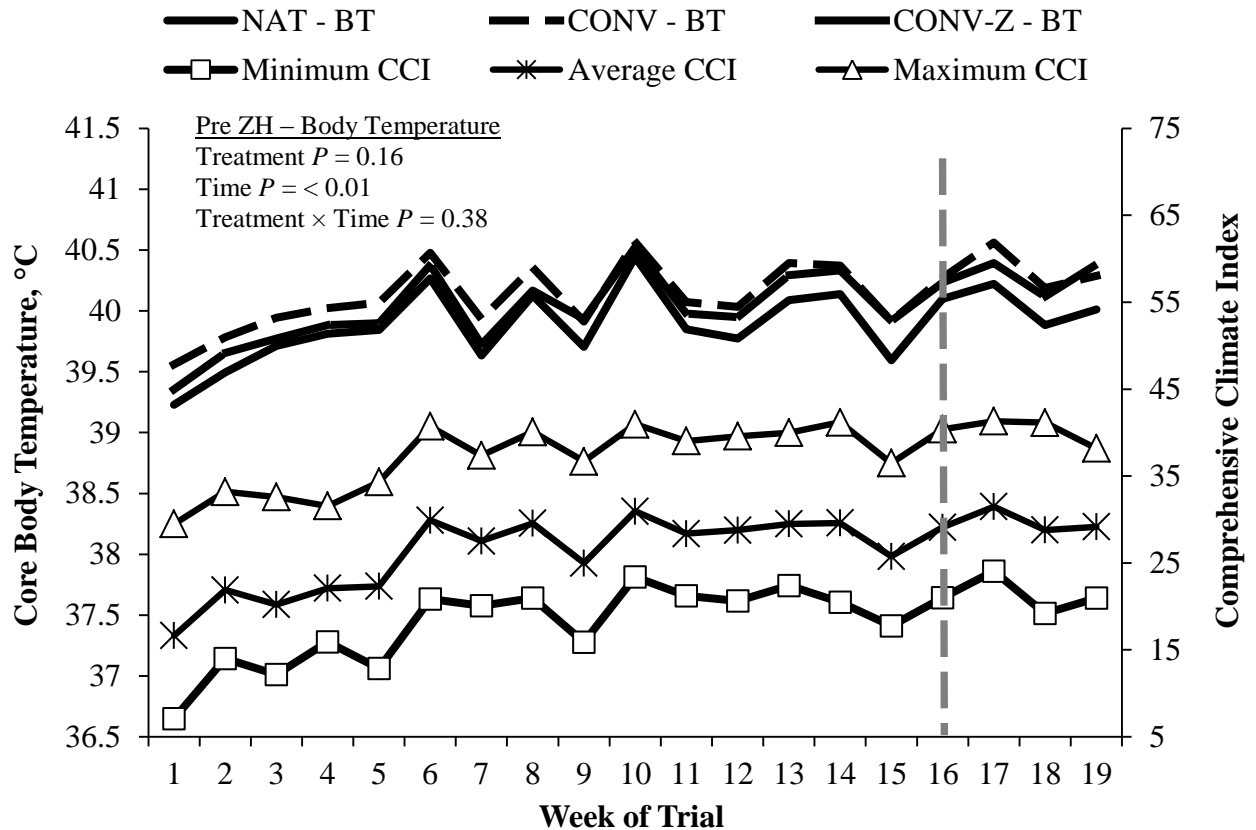


Figure 5.1. The effect of growth-promoting technologies on average body temperature (BT) prior to the ZH feeding period and corresponding comprehensive climate index (CCI) results. Treatments include: 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z). Body temperature was measured by a remote monitoring device (rumen bolus) that recorded temperature every 3 min. Average daily BT were summarized by week prior to the zilpaterol hydrochloride (ZH) feeding period. Week 16 represents the start of supplementing ZH. Average weekly BT ranged from 39.4 to 40.5°C, with a mean of 40.0°C. Treatment did not have an effect on average BT prior to supplementing ZH ( $P = 0.16$ ). Average BT did vary over time in conjunction with changes in average CCI ( $P < 0.01$ ). Data presented as LSM.

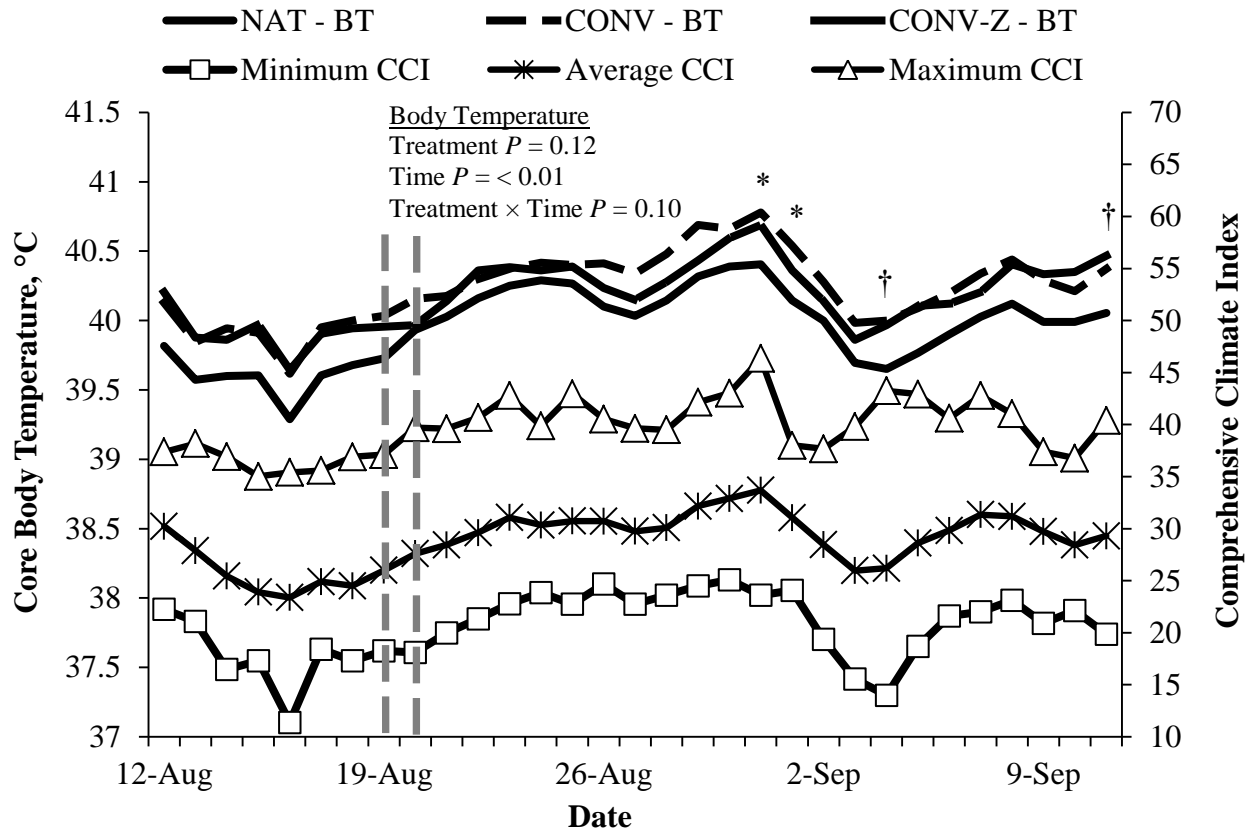


Figure 5.2. The effect of growth-promoting technologies on average body temperature (BT) during the ZH feeding period and corresponding comprehensive climate index (CCI) results. Treatments include: 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z). Body temperature was measured by a remote monitoring device (rumen bolus) that recorded temperature every 3 min. Data are presented as average daily BT. August 19<sup>th</sup> (n = 4) and 20<sup>th</sup> (n = 2) represent the initiation of ZH supplementation (fed for 20 d with a 3-4 d withdrawal). A tendency ( $P = 0.10$ ) for a treatment  $\times$  time interaction was detected. \* represents NAT steers had a lower BT than CONV steers ( $P < 0.05$ ) and CONV-Z steers being intermediate and not different. † Represents NAT steers had a lower BT than CONV and CONV-Z steers ( $P < 0.05$ ). Data presented as LSM.

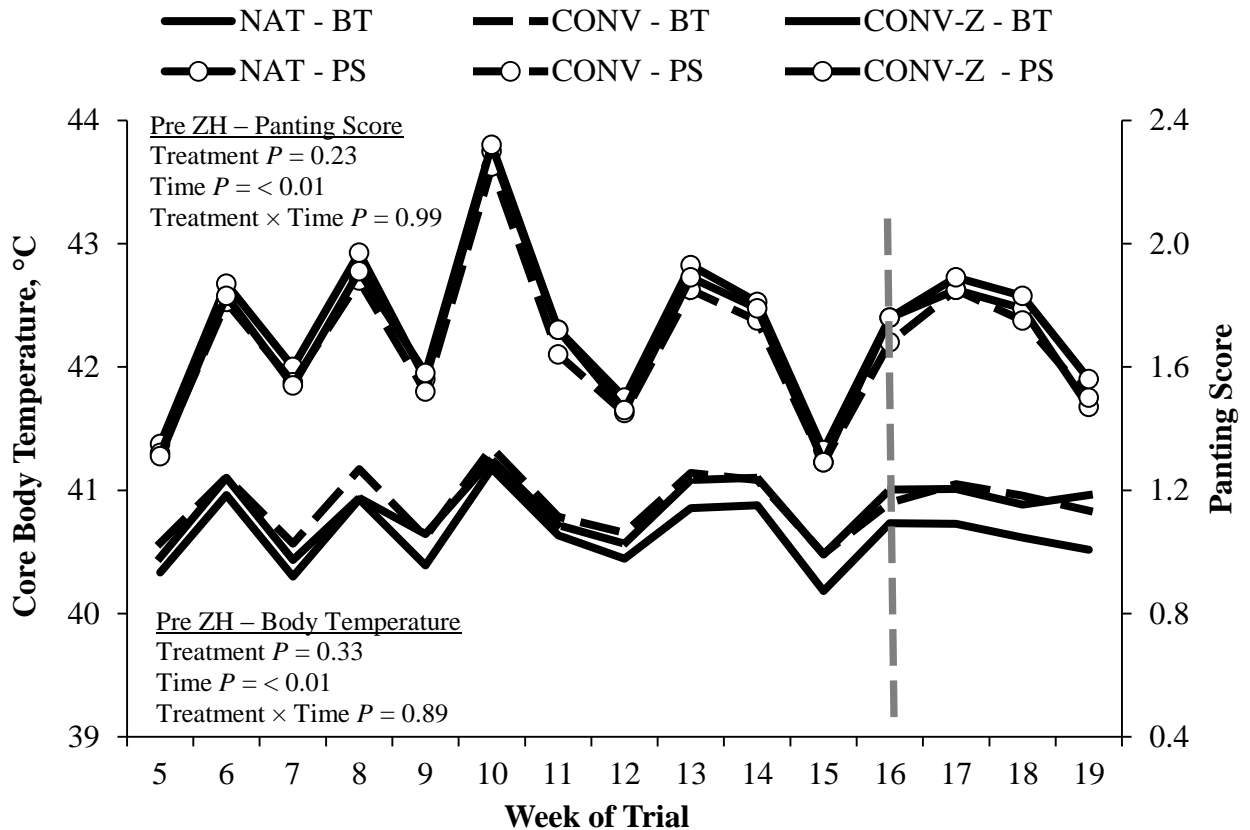


Figure 5.3. The effect of growth-promoting technologies on average evening body temperature (BT) and panting score (PS) prior to the ZH feeding period. Treatments include: 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z). Body temperature was measured by a remote monitoring device (rumen bolus) that recorded temperature every 3 min. Evening BT was averaged from 1630 to 1830 h and summarized by week prior to the zilpaterol hydrochloride (ZH) feeding period. Evening PS was observed from 1700 to 1800 h. Cattle were assigned a PS of 0-4: 0 = normal respiration; 1 = elevated respiration; 2 = moderate panting and/or presence of drool or small amount of saliva; 3 = heavy open-mouthed panting; saliva usually present; 4 = Severe open-mouthed panting accompanied by protruding tongue and excessive salivation; usually with neck extended forward (Mader et al., 2006). Week 16 represents the start of supplementing ZH. Average weekly evening BT ranged from 40.0 to 41.3°C, with a mean of 40.7°C. Treatment did not have an effect on evening BT ( $P = 0.33$ ) or PS ( $P = 0.23$ ) prior to supplementing ZH. Average BT did vary over time in conjunction with changes in average CCI ( $P < 0.01$ ). Data presented as LSM.

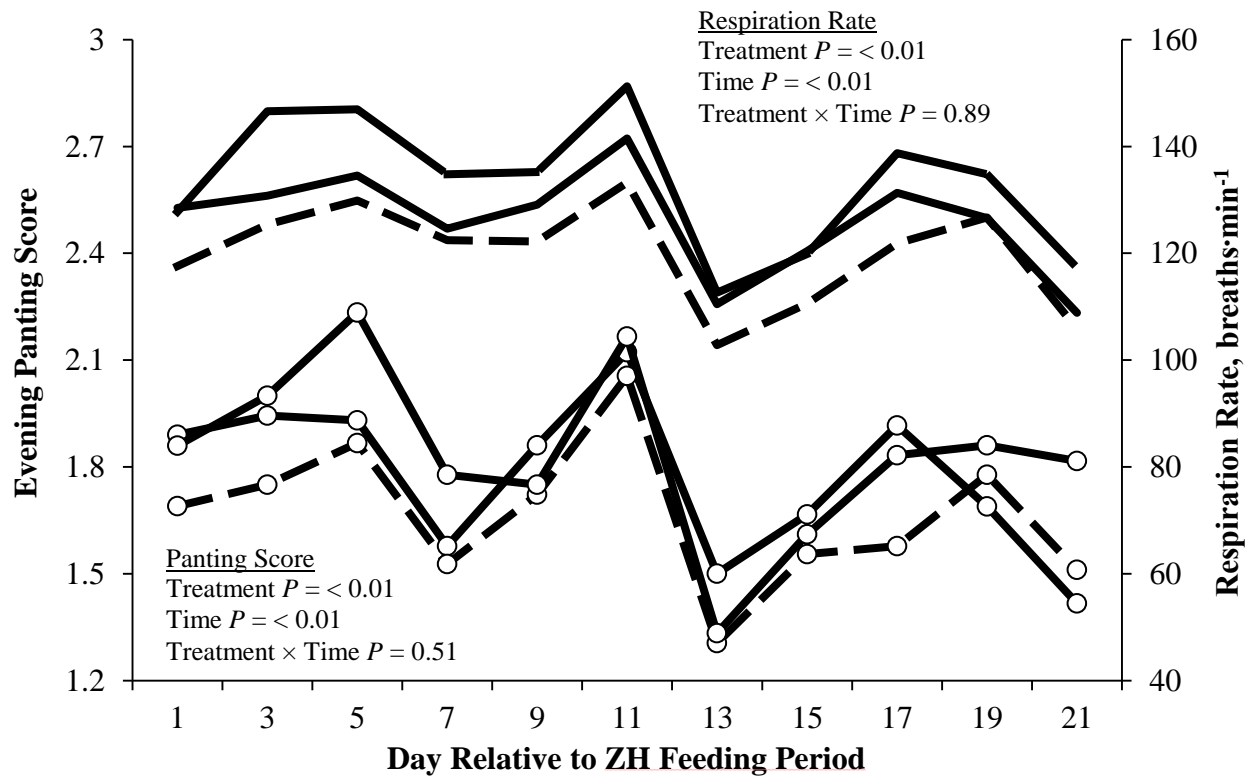
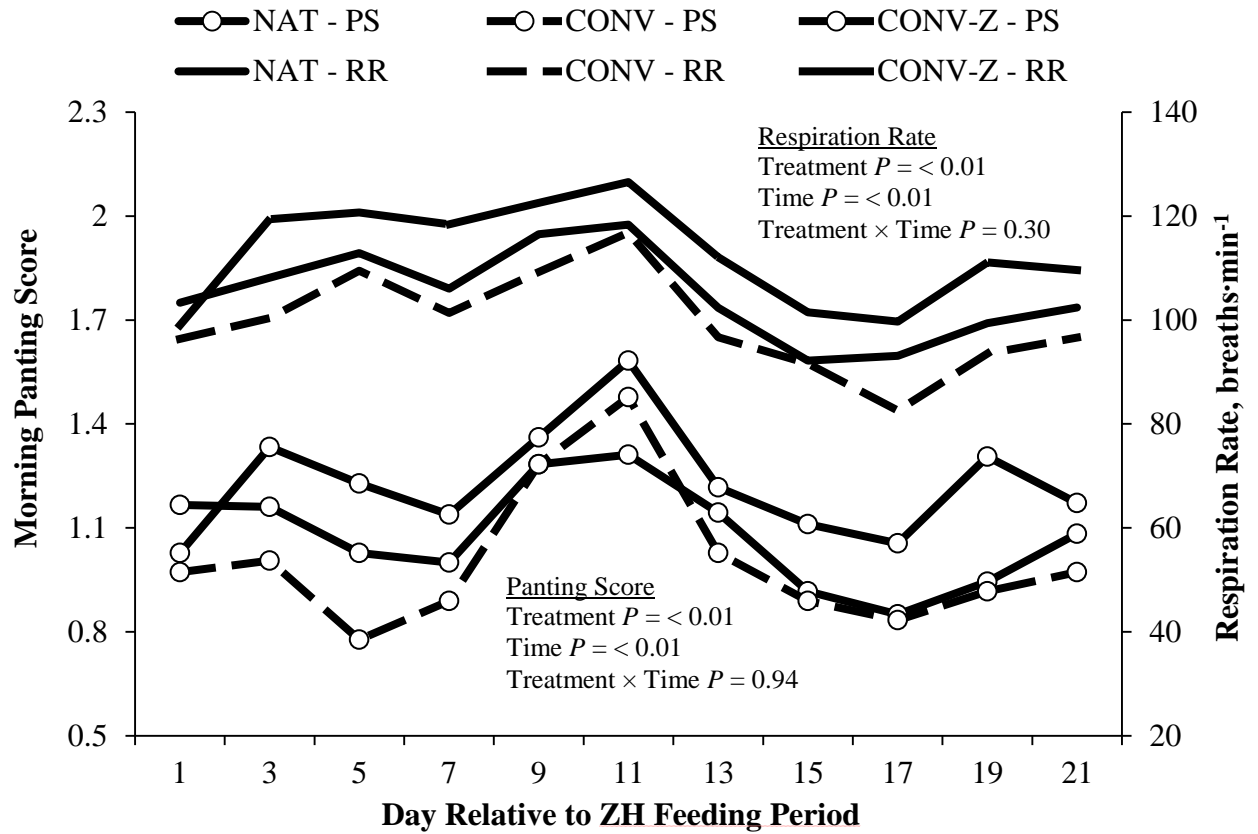


Figure 5.4a. The effect of growth-promoting technologies on average morning panting score (PS) and respiration rate (RR) during the ZH feeding period. Treatments include: 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z). Morning PS and RR were collect from 1000 to 1100 h every other day. Cattle were assigned a PS of 0-4: 0 = normal respiration; 1 = elevated respiration; 2 = moderate panting and/or presence of drool or small amount of saliva; 3 = heavy open-mouthed panting; saliva usually present; 4 = Severe open-mouthed panting accompanied by protruding tongue and excessive salivation; usually with neck extended forward (Mader et al., 2006). Morning PS was increased in CONV-Z steers versus the CONV and NAT steers (1.23 vs. 1.00 and 1.08;  $P < 0.01$ ). Morning RR was different across all 3 treatment ( $P < 0.01$ ), being greatest for CONV-Z steers (112.8), intermediate for NAT steers (105.0), and the lowest for CON steers (99.5). Data presented as LSM.

5.4b. The effect of growth-promoting technologies on average evening panting score (PS) and respiration rate (RR) during the ZH feeding period. Treatments include: 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z). Evening PS and RR were collect from 1700 to 1800 h every other day. Cattle were assigned a PS of 0-4: 0 = normal respiration; 1 = elevated respiration; 2 = moderate panting and/or presence of drool or small amount of saliva; 3 = heavy open-mouthed panting; saliva usually present; 4 = Severe open-mouthed panting accompanied by protruding tongue and excessive salivation; usually with neck extended forward (Mader et al., 2006). Morning PS was increased in CONV-Z versus the CONV steers (1.84 vs. 1.67;  $P < 0.01$ ), and NAT were not different from either treatment (1.74). Morning RR was different across all 3 treatment ( $P < 0.01$ ), being greatest for CONV-Z steers (133.8), intermediate for NAT steers (125.8), and the lowest for CON steers (120.1). Data presented as LSM.

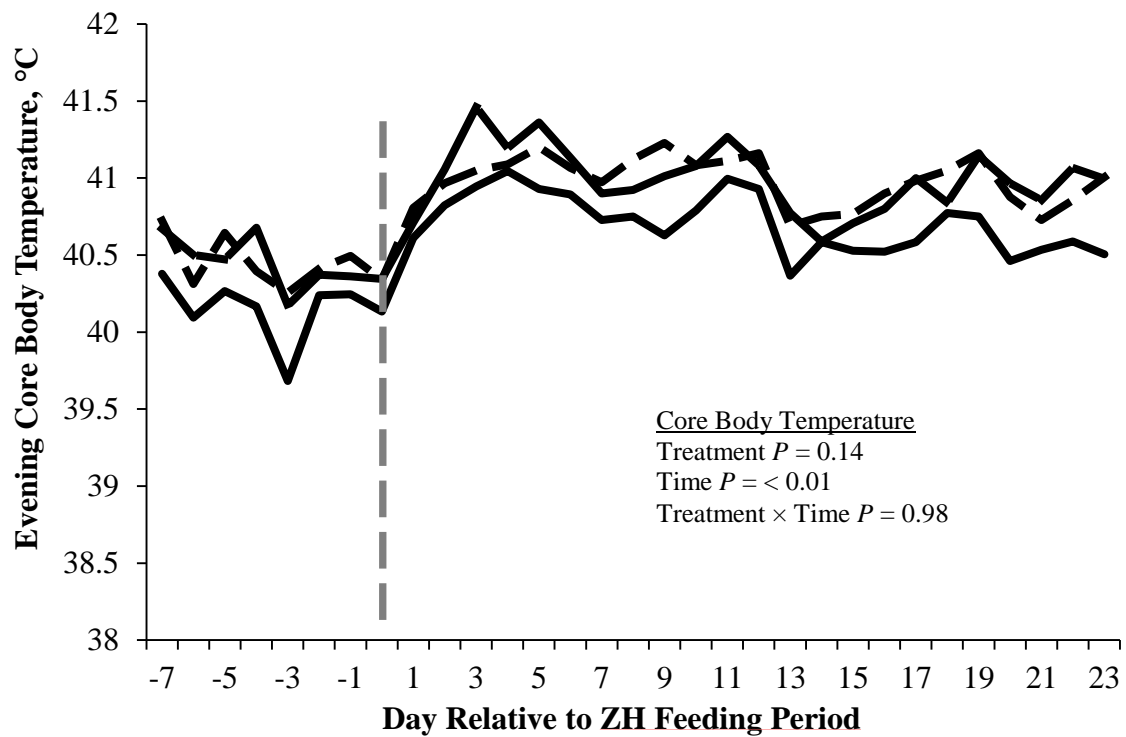
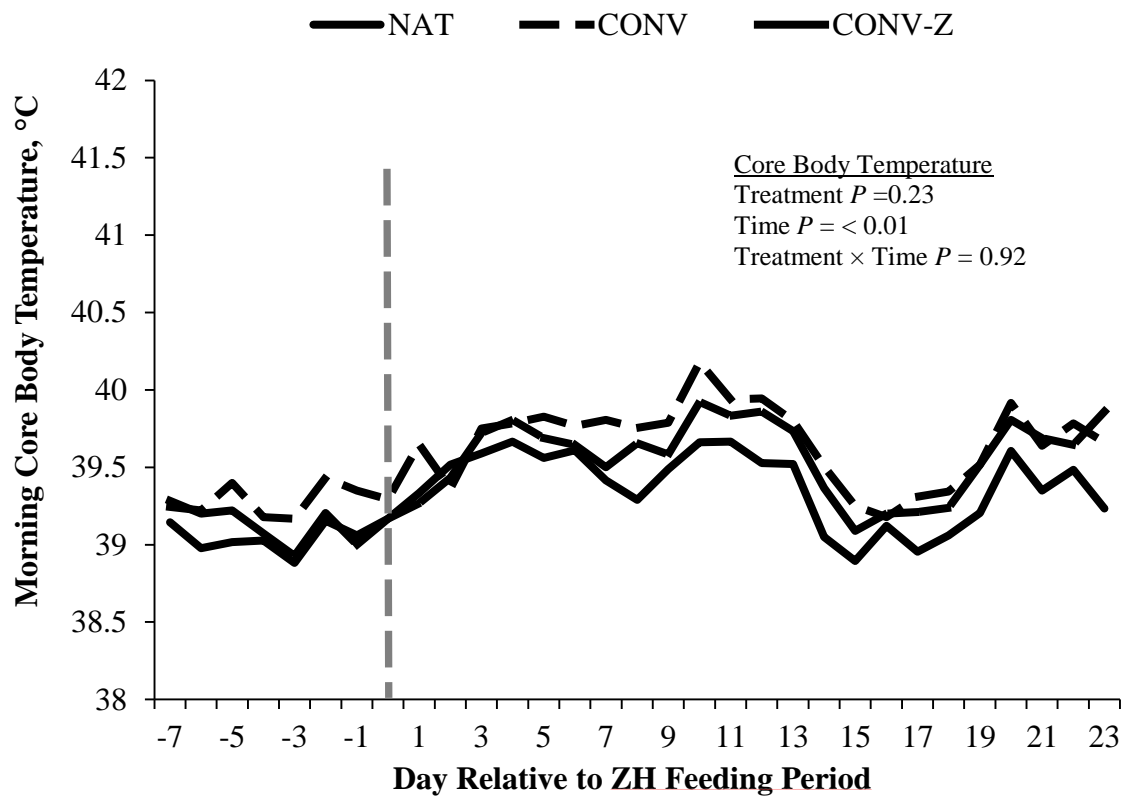


Figure 5.5a. The effect of growth-promoting technologies on average morning body temperature (BT) during the ZH feeding period. Treatments include: 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z). Body temperature was measured by a remote monitoring device (rumen bolus) that recorded temperature every 3 min. Morning BT was averaged from 0930 to 1130 h every day during the zilpaterol hydrochloride (ZH) feeding period. These times corresponded to 30 min prior and 30 post the collection of morning PS and RR data that is presented in figure 5.4a. Average morning BT ranged from 39.1 to 39.9, with a mean of 39.5°C. Treatment did not have an effect on average morning body temperature prior to supplementing ZH ( $P = 0.23$ ). Data presented as LSM.

5.5b. The effect of growth-promoting technologies on average evening body temperature (BT) during the ZH feeding period. Treatments include: 1) Natural – no antibiotics, ionophores, growth implants or beta-agonists (NAT), 2) Conventional – fed tylosin, monensin, received growth implant, no beta-agonist (CONV), 3) Conventional w/ zilpaterol – fed tylosin, monensin, received growth implant, fed zilpaterol hydrochloride (87.6 mg/steer last 20 DOF; CONV-Z). Body temperature was measured by a remote monitoring device (rumen bolus) that recorded temperature every 3 min. Evening BT was averaged from 1630 to 1830 h every day during the zilpaterol hydrochloride (ZH) feeding period. These times corresponded to 30 min prior and 30 post the collection of evening PS and RR data that is presented in figure 5.4b. Average evening BT ranged from 40.3 to 41.2°C, with a mean of 40.9°C. Treatment did not have an effect on average evening BT during the ZH supplementation period ( $P = 0.14$ ). Data presented as LSM.

## **APPENDIX**

All procedures involving live animals were approved by the Oklahoma State University Institutional Animal Care and Use Committee

Protocol # AG 12-2

## VITA

Bryan Christopher Bernhard

Candidate for the Degree of

Doctor of Philosophy

**Thesis: EFFECTS OF GROWTH-PROMOTING TECHNOLOGIES ON BEHAVIOR,  
MOBILITY, HEALTH PARAMETERS AND HEAT STRESS OF FINISHING STEERS**

Major Field: Ruminant Nutrition

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Animal Science at Oklahoma State University, Stillwater, Oklahoma in July, 2014.

Completed the requirements for the Master of Science in Animal Science at Texas Tech University, Lubbock, Texas in December, 2011.

Completed the requirements for the Bachelor of Science in Animal Science at Texas Tech University, Lubbock, Texas in May, 2009.

Experience:

Graduate Research Assistant and Assistant Livestock Judging Coach –  
Texas Tech University (2009-2011)

Graduate Research and Teaching Assistant – Oklahoma State University  
(2012-2014)

Professional Memberships: American Society of Animal Science; American  
Simmental Association; American Boer Goat Association