

FEEDING TRIALS ANALYZING THE PRODUCTION EFFECTS OF
DIETARY ADDITIVES IN GROWING CATTLE

by

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ABSTRACT

In the first study, thirty-two Hereford x Simmental steers (250.0 ± 15 kg **Initial BW**) were assigned to four dietary treatments in a completely randomized design (4 steers/pen, 2 pens/treatment) in order to evaluate the effects of supplementing Monensin (**MON**; Rumensin; Elanco Animal Health, Greenfield, IN) in comparison and in combination (**CMB**) with a proprietary Probiotic blend (**PRO**; MultSacch®, Biomart Nutrição Animal Imp. and Exp. Ltda.) on the growth performance, feed efficiency, and on cost of gain. Individual feed intake was monitored using a GrowSafe System (GrowSafe Systems Ltd. Calgary, AB, Canada) and weights were recorded weekly for a 90 d growth data period. Supplementation with PRO and CMB both increased Gain:Feed (G:F) by 6% with respect to control (**CON**) steers ($P = 0.01$). No treatment effects were detected in initial body weight (IBW), final body weight (FBW), or average daily gain (ADG) ($P > 0.05$). DMI was lowest in CMB steers ($P = 0.04$), and this same group had the lowest cost of gain ($P < 0.001$). Our findings indicate that when compared with MON, PRO seems to be just as effective in stabilizing intraruminal milieu and increasing ruminal fiber utilization when steers are fed rapidly fermentable carbohydrate grain-based diets, thus improving feed efficiency without the need of MON supplementation. However, combined use of PRO and MON results in the lowest cost of gain ($P = 0.03$). Further experimentation is required to ascertain the effect substituting MON with different mixtures of probiotics will have long term on cattle growth performance and profitability.

The second study consisted in feeding Red Angus steers ($n=24$; 260 ± 25 kg) in order to analyze the effects of supplementation of zilpaterol hydrochloride (ZH) under heat stress conditions on respiration rate (RR), rectal temperature (RT), growth performance (GP), Biological Impedance Analysis (BIA), carcass traits (CT), and complete blood count (CBC). Steers were randomly assigned to a 2 x 2 factorial treatment arrangement ($n=6$ /group) with

factors including heat stress (HS; Temperature Humidity Index (THI) = 73 to 85) or thermal neutral (TN; THI=68) conditions and with/without supplementation of ZH (0 or 8.38 mg/kg/d on 88% DM basis). Steers were provided 9 d to acclimate to tie stalls rooms under TN conditions before starting the study. TN steers were pair-fed to the average daily dry matter intake (DMI) of HS steers. *Ad libitum* water intake (WI) was recorded daily. HS and TN steers were harvested on d 22 and 23, respectively. By design, DMI did not differ between environment or supplement groups ($P>0.31$). Initial body weight did not differ between environments or supplement groups ($P>0.62$). RT, RR, and WI were greater ($P<0.01$) in HS steers compared to TN steers. There was a supplement by environment interaction ($P=0.02$) for RT, as HS steers fed ZH had lower RT than HS control steers (39.1 vs 39.5 °C, respectively). Average Daily Gain (ADG) was 20% higher ($P=0.04$) in HS steers compared to TN steers. BIA phase angle (PA), resistance (R), and reactance (R_c) values were significantly lower in HS steers by 19%, 8.5%, and 25%, respectively ($P \leq 0.04$). CT and CBC did not differ due to environment, treatment, or environment by treatment interactions ($P>0.05$). Our results suggest that feedlot steers under our experimental conditions display some sensitivity to HS through GP, RR, RT, and BIA; however, this does not translate to a significant impact on CT or CBC. Furthermore, ZH supplementation did not appear to impact animal well-being negatively.

CHAPTER 1 – LITERATURE REVIEW

Introduction

Major strides taken in recent decades in the improvement of growth efficiency in beef cattle can largely be attributed to two types of growth enhancement technologies: ionophores and beta-adrenergic agonists. Ionophores are antibiotics used to modulate rumen fermentation and lower incidence of coccidiosis in growing cattle. Beta-adrenergic agonists (α AA) are non-hormonal compounds that are supplemented to cattle during the final stage of the feeding period in order to prioritize the development of skeletal muscle over the development of fat so as to improve lean muscle growth. Despite these desirable outcomes in efficiency noticed through supplementation of these additives, there are several external factors that influence and / or dictate their use in cattle feeding operations. Ionophore supplementation of cattle has provoked much criticism and skepticism in recent years among some consumers due to a perceived, and unfounded, worry of antibiotic resistance emerging in humans who consume beef from ionophore supplemented cattle. Consumer preferences in this case have prompted some producers to look for alternatives to ionophores so as to maintain feed efficiency without compromising the marketability of their beef. Essential oils, prebiotics, and probiotics are some of the alternatives to ionophores currently being evaluated. In the case of β AAs, anecdotal accounts in 2013 of cattle supplemented the β AA zilpaterol hydrochloride (ZH) having high mortality rates and incidence of lameness when transported to slaughtering facilities, particularly during the summer months, resulted in the manufacturer voluntarily pulling ZH from the American market over concerns of ZH supplementation negatively affecting animal well being. Thus, the objective of this research is focused on investigating the effects of growth enhancement technologies in growing cattle in comparison to an alternative (probiotic) and under

heat stress conditions (ZH). In the first study we evaluated the effects on growth efficiency of supplementation of a probiotic blend in comparison to monensin. The second study consists in analyzing the effects of supplementation of ZH under HS conditions on growth performance, carcass characteristics, and body composition.

Cattle feeding / production

Industry Overview

Demand for beef is rising globally due to an increasing human population and growing per capita consumption of beef in ‘developing’ countries (USDA, 2016; OECD/FAO, 2018). This means cattlemen must continue to strive for implementing efficient management practices in order to be able to meet the projected rise in demand of beef. The avenues through which this demand is expected to be met varies between countries. Brazilian meat production is expected to grow thanks to an abundance of natural resources and improvements in feeding strategies whereas Chinese producers’ growth is expected to come from growing economies of scale where small livestock production units become larger more efficient enterprises (OECD/FAO, 2018). The United States’ animal agriculture sector is expected to benefit from high slaughter weights that are a result of several factors such as genetic improvements, decreasing feed costs (allows longer feeding period), and the implementation of several growth enhancements technologies (USDA, 2016; OECD/FAO, 2018). Historically speaking, the American beef industry has been able to capitalize on increased demands of beef thanks to the utilization of growth promotant technologies. This has positioned the American beef industry as a leader in terms of productivity per animal in not only the beef sector but animal agriculture in general (Drouillard, 2018).

Recent Trends

The beef sector has always distinguished itself from other branches of animal agriculture (swine production, poultry etc.) in that it remains a highly horizontally segmented industry unlike the other forms of animal agriculture listed. These differences in consolidation are largely due to the differences in gestation period, growth duration, and land requirements, among many other variables, that exist in the beef sector making vertical integration more challenging if not inconvenient; however, the beef industry is becoming what is known as more vertically aligned (Drouillard, 2018). Vertical alignment means greater cooperation between the different segments so as to improve either management practices that if done incorrectly in one segment can incur losses on another or in order to satisfy end consumer preferences. Newfound interest among consumers in the content of the feed or the implementation of technologies applied to cattle is driving many of these branded programs further encouraging alignment between different segments of the beef industry in order for beef to qualify as being listed in the program of interest and to satisfy consumer demands of transparency. Consumer preferences; however, do not always align with the conventional rearing practices of the industry causing shifts in certain aspects of cattle feeding (Pearson and Dutson, 1991).

Sustainability

Cattle play an important role in maintaining the sustainability of the human food supply. A study that examined the potential effects of eliminating U.S. animal agriculture on the environment and food supply determined that the total greenhouse gases emitted in the U.S. would decrease by 2.6% (drop in 0.36% of global emissions) after taking into account the emissions of the plant-based alternative to the current food supply, but the authors of this study found that the alternative food supply would result in shortages in essential dietary nutrients

(White and Hall, 2017). This relatively low-impact on environmental footprint and serious risk of insufficient supply of essential nutrients is largely due to the role of ruminants, and cattle in particular, in converting human-inedible plants to a nutritious human-edible product, beef. Cattle are ruminants which means they are able to digest polymers such as cellulose (inedible to humans) by breaking them down into monomers that are then metabolized into volatile fatty acids that serve as sources of energy for the animal (Cummings and Macfarlane, 1997). Although conventional beef cattle systems in the U.S. ‘finish’ cattle on predominantly grain-based diets, research by the National Academies of Sciences, Engineering, and Medicine (2016) concluded that the composition of the feed consumed in a lifetime of conventionally raised beef cattle in the U.S. is 10% grain and 90% human-inedible plants. This makes the U.S. beef industry a necessary component of a highly efficient food supply that utilizes resources that are of no dietary value to humans.

Beta-adrenergic agonists

Adrenergic Receptors and Agonists

Adrenaline (Epinephrine) and noradrenaline (Norepinephrine) are catecholamines that act as neurotransmitters that affect central nervous system regulation of eating behavior and/or upregulate/downregulate several metabolic pathways in peripheral tissues (Scanzano and Cosentino, 2015). These catecholamines initiate these responses when they bind to what are known as adrenergic receptors. Adrenergic receptors are part of the G-protein coupled receptor superfamily. Receptors in this family are characterized by having seven transmembrane receptors on the surface of varying mammalian cell types such as skeletal muscle, adipose, and cardiac tissue (Lefkowitz et al., 1983). Adrenergic receptors are classified into three main types α_1 , α_2 , and β , which can each be divided into several subtypes (Lefkowitz et al., 1983). Successful

binding of ligands to these receptors initiates a cascade of events known as the cyclic adenosine monophosphate (cAMP) signal pathway. Following ligand activation of the receptor, the enzyme adenylyl cyclase is activated and converts adenosine triphosphate (ATP) to cAMP. Cyclic AMP is a secondary messenger that relays the extracellular signal to the cytosolic enzyme protein kinase A, which then activates several metabolic enzymes via phosphorylation (Scanzano and Consentino, 2015). This section of the review will focus on analyzing a specific type of synthetic β AA known as zilpaterol hydrochloride (ZH; Zilmax) that is supplemented to beef cattle in order to promote muscle growth and feed efficiency.

Beta-adrenergic agonists as a growth enhancing agent in cattle

The subtypes of β -adrenergic receptors (β ARs) are β_1 , β_2 , and β_3 . The distribution of these receptors in tissues and organs varies between species. Cattle have been determined to predominantly express β_2 -adrenergic receptors in skeletal muscle (>99%) and adipose tissue (>90%) while β_1 is more prevalent in bovine cardiac tissue (Zheng et al., 2005). The three types of beta-adrenergic receptors function similarly when activated by either the natural occurring agonists (Adrenaline and Noradrenaline) or a synthetic pharmacological agonist (Zheng et al., 2005). Beta-adrenergic agonists that are orally ingested in livestock species such as cattle initiate the previously outlined intracellular cascade of events that results in the activation of metabolic enzymes such as hormone-sensitive lipase. Hormone-sensitive lipase hydrolyzes triglycerides into free fatty acids, and this subsequently inhibits *de novo* biosynthesis of fatty acids (Zheng et al., 2005; Mersmann, 1998). The free fatty acids serve as sources of energy for muscle cells that begin to increase protein accretion and downregulate lipogenesis and proteolytic activity (Mersmann, 1998). Historically, the first use of β AA supplementation in cattle occurred with the β_2 AA Clenbuterol (McMillan et al., 1992). Clenbuterol supplementation in cattle displayed

promising nutrient repartitioning activity due to its ability to elicit metabolic responses associated with muscle accretion (McMillan et al., 1992); however, clenbuterol use was subsequently banned in the United States due to concerns over residue effects on human health. Since then, there are only two β AAs approved for use in the U.S., which are ractopamine hydrochloride and ZH. A meta-analysis analyzing several dozen cattle feeding trials that used either ZH or RH determined that ZH supplementation resulted in greater rate of gain, Longissimus muscle area, increased gain:feed (Lean et al., 2014). Ractopamine supplementation on the other hand did not reduce fat thickness and resulted in better tenderness scores than ZH (Lean et al., 2014). A publication by Capper and Hayes (2012) quantified the contribution of RH and ZH supplementation to growth performance and carcass characteristics from numerous cattle feeding trials, which yielded a mean central tendency of an 18% increase in growth rate and a 0.5% increase in dressing percentage in β AA supplemented cattle. Capper and Hayes (2012) then determined that eliminating β AA supplementation in beef cattle would require an 11.8% increase in the number of cattle slaughtered in order to supply the same amount of beef. In summary, the signaling of β AR in cattle sets off a cascade of cellular events that results in the degradation of adipose tissue in order to increase muscle synthesis. These repartitioning agent functionalities contribute to ZH's popularity among producers and the impact its removal from the market has had on the beef industry in terms of volume of production and environmental emissions.

Zilpaterol hydrochloride concerns

Concerns regarding the interacting effects of heat stress and ZH supplementation have arisen in recent years due to the anecdotal accounts that lead to the removal of ZH from the market. This perception is founded on the understanding that both heat stress and ZH

supplementation stimulate \square ARs (Swanson et al., 2019). This leads to the suspicion that supplementing \square AA during the summer months is contributing to a compounding of the harmful effects on animal well-being that are normally associated with heat stress (Grandin, 2013). The research investigating this association in beef cattle is limited; however, there have been several studies investigating the interacting effects of \square AA and HS in sheep. These studies have not noticed any harmful effects on effects on well-being but similarly the growth improvements and carcass merit normally associated with \square AA supplementation have not been noted either (Marcías-Cruz et al., 2010; Gibbs et al., 2019). This has caused some speculation that the effects of HS nullify any benefit from \square AA supplementation, which would make \square AA supplementation during the summer cost ineffective.

Heat Stress

Defining Environmental Stress

Desirable production performance in cattle requires cattle to be in what is known as the thermal neutral zone. The thermal neutral zone is the area where cattle are neither forced to produce extra heat or dissipate heat in order to maintain thermostatic homeostasis. Cattle generate metabolic heat by increasing dry matter intake when they are exposed to lower critical temperatures (between -4 and 2°C). On the other hand, cattle begin to dissipate heat through various methods when they are exposed to temperatures at or above the upper critical limit (26°C). Thus, the thermal neutral zone is defined as the area between the lower and critical temperatures.

Temperature Humidity Index

Although temperature is a defining characteristic of the boundaries at which cattle productivity is affected, it is by no means the only variable that is considered when determining

an animal's susceptibility to environmental stress. Humidity, solar radiation, age, weight, purpose (lactation vs muscle growth), and many other variables can either lower or increase the threshold at which thermostatic homeostasis is lost (Australian Livestock Corporation, 2020). In order to better visualize the ranges at which productivity is affected, scientists have developed what is known as the Temperature-humidity index (THI). The THI is derived from measured effects of temperature and humidity in specific types of production settings (Australian Livestock Corporation, 2020).

Physiological Responses

Cattle enter a state of heat stress when the core body temperature exceeds the normal specified range and when the total heat load exceeds the rate at which they are dissipating heat (Bernabucci et al., 2010). There are two strategies for dissipation of heat termed sensible (via thermal gradient) and insensible (latent) heat loss. Sensible heat loss consists in dissipation of heat through conduction, convection, and/or radiation. These methods of heat dissipation become ineffective when environmental temperatures exceed body temperature, therefore, the primary methods of heat dissipation under heat stress conditions are through the insensible heat loss methods: sweating and panting (Maia and Loureiro, 2005). Under high humidity conditions, sweating is considered ineffective due to lack of a vapor pressure gradient. Panting (respiration rate) is consequently increased above the normal range of 20-50 breaths per minute in order to be able to help the animal dissipate heat (Thomas and Pearson, 1986). This increased rate of panting results in an increased loss in carbon dioxide, which results in respiratory alkalosis due to an imbalance in the carbon dioxide to bicarbonate ratio. Cattle then excrete bicarbonate through their urine in order to correct this imbalance and simultaneously, salivary bicarbonate is being lost due to drooling (Bianca, 1965). This presents a new challenge because depletion of

bicarbonate, a buffering agent of the rumen, results in reduced ruminal pH, leading to ruminal acidosis (Bandaranayaka and Holmes, 1976).

Production Significance

The increased maintenance requirements caused by the physiological responses to heat stress has detrimental effects on growth performance (Bernabucci et al., 2010). Decrease in growth performance is largely attributed to increased mortality rate and hypophagia (decreased DMI). Depressing feed intake lowers the amount of metabolic heat being produced but has negative consequences on production. Decreased DMI causes cattle to enter a negative energy balance (NEB) and this weakened condition increases its susceptibility to several diseases. Something producers do to prevent cattle from entering a NEB is increase the energy content (grain) of the ration (Bernabucci et al., 2010). However, the potential for cattle to experience ruminal acidosis under heat stress is already significantly higher due to the base:acid imbalance that was previously mentioned to be related to respiratory rate; so increasing energy content in the ration further complicates the safeguarding of animal well-being under heat stress and thus increases the loss in profitability. Heat stress has been estimated to cost the livestock industry as a whole between \$1.9 and \$2.7 billion per year (St-Pierre et al., 2003). More precisely, among beef cattle feeders this impact has been estimated to constitute losses of about \$78/hd (Belasco et al., 2015). Producers and researchers are beginning to pay closer attention to potential methods for mitigating the effects heat stress has on cattle performance in order to prepare for worsening conditions and increasing susceptibility of cattle to heat stress.

Management and Mitigation

The Intergovernmental Panel on Climate Change (IPCC) (2018) reports that unless annual emissions are reduced by about a half by 2030, then we can expect warming greater than

1.5°C . This projected increase in temperature means any loss in production in cattle being incurred right now due to the effects of heat stress are expected to increase. The effects of these unfavorable climatic conditions are expected to be compounded by an increasing susceptibility to heat stress conditions that is ironically being indirectly selected for by breeders. As stated previously, metabolic heat generated by cattle is one of the main drivers of increased body temperature that eventually overwhelms the dissipation mechanisms. Highly productive cattle (e.g kilograms of muscle growth or liters of milk produced) generate more metabolic heat than less productive cattle meaning producers are indirectly lowering the threshold to heat stress tolerance in their cattle (Renaudeau et al., 2012).

Feed additives

Monensin

Monensin is an ionophore that is classified as a polyether antibiotic that is incorporated in the ration fed to growing cattle in order to increase growth and feed efficiency and to serve as a coccidiostat agent (Russell and Strobel, 1989). Monensin targets gram-positive bacteria in the rumen that inhibit efficient ruminal fermentation and improves efficiency in fermentation by modulating methanogenesis, selective volatile fatty acids (VFA) production, increasing pH, and prevention of coccidiosis incidence (Schelling, 1984). Methanogenesis is an energy consuming process that results in the formation of an undesirable product from an environmental standpoint, methane (Knapp et al., 2014). Monensin decreases methane production by about 4-31% and in doing so promotes an energy favorable process, which is the production of the VFA propionate over acetate (Schelling, 1984). Propionate is considered to be the more favorable VFA in ruminal fermentation due to it being more efficiently utilized by the ruminant (Richardson et al., 1976). Volatile fatty acids arising from ruminal fermentation are the primary energy source for the

ruminant thus any improvement in VFA efficiency translates to an overall growth and feed efficiency (Russell et al., 1992). A meta-analysis conducted by Duffield et al. (2012) that focused on analyzing the results of several dozen cattle feeding trials that evaluated the effectiveness of monensin noticed a 6.4% improvement in feed efficiency in monensin supplemented cattle.

Monensin increases ruminal pH, which is favorable in production settings that normally feed pH lowering, high grain diets that can cause ruminal acidosis. Another significant contribution of monensin to cattle feeding is through its function as a coccidiostat that prevents the costly and deadly disease coccidiosis (Noack et al., 2019). Despite these improvements in growth and feed efficiency, that have positive environmental implications, use of monensin is being targeted due to monensins classification as an antibiotic. A recent survey noted an increased willingness among consumers to pay a premium for beef from cattle that were never treated with antibiotics (Rummo, 2016). This, however, does not take into account the differences in classification between antibiotics since monensin is not a therapeutic antibiotic and has no relevance in human medicine making its use in cattle feeding harmless.

Probiotics

Yeasts are eukaryotic, single celled organisms that are part of the fungi family. Supplementation of probiotics has been considered an effective method for stabilizing the intraruminal milieu, improving fermentation in ruminants fed grain diets, increasing nutrient digestibility, and increasing overall feed and growth efficiency (Silva et al., 2018; Chaucheyras-Durand et al., 2012). One species of yeast commonly incorporated in ruminant diets and in Probiotic blends is *Saccharomyces cerevisiae* (SC). *Saccharomyces cerevisiae* live cells have been reported to have the ability to remove oxygen from the rumen environment and release

essential enzymes, vitamins and other nutrients or growth factors, which could facilitate bacteria to have a high viability and activity in the rumen (Ghazanfar et al., 2017; Newbold et al., 1993).

Beef Carcass Composition

Beef grading history and overview

The U.S. standards for dressed beef were first formulated in 1916 and since then have been amended based on stakeholder input and the development of technologies that assess specific characteristics of the carcass (USDA, 2017). The two categories in which a beef carcass is assessed are yield and quality grades. Yield grades (Range 1-5) are estimates of the amount of boneless trim and excess fat on a carcass (Hale et al., 2013). A yield grade 1 (YG1) is considered to have a higher proportion of lean meat on a carcass (high cutability) whereas a yield grade 5 (YG5) is indicative of a higher proportion of fat on a carcass (low cutability). The factors used to determine yield grade are: 1. hot carcass weight 2. the amount of kidney, pelvic, and heart fat 3. the longissimus muscle area 4. 12th rib fat thickness and overall external fat covering. Quality grade on the other hand is a measure of the palatability of the meat. The palatability of meat (tenderness, juiciness, and flavor) is considered to be affected by two measurable characteristics of the carcass, which are maturity and marbling (Hale et al., 2013). Marbling is the amount of intramuscular fat on the carcass and is assessed on the longissimus muscle or “ribeye” by cutting between the 12th and 13th rib of the carcass. Marbling scores range from Standard, Select, Choice, and Prime with Prime meaning a higher degree of marbling and Standard a low degree of marbling. Subtypes exist in each marbling score and are denoted by $\bar{}$, $^{\circ}$, and $^{+}$ with $\bar{}$ being lower degrees of marbling and $^{+}$ a higher degree of marbling. The second component of a quality grade, maturity, is assessed by analyzing ossification of the cartilage, color, texture, and bone characteristics all of which are indicators of physiological age of the animal. Maturity scores

range from A-E with A meaning a younger animal (9-30 months) and E an older more mature animal (>96 months).

Muscle Biology / Meat Science

Individual skeletal muscle fibers are composed of several different subunits. The first of these subunits is known as the myofibril, which consists in long filaments that run parallel to each other in order to form the muscle fiber. The sarcomere is the smallest functional unit of skeletal muscle fibers and it is composed of contractile proteins known as myofilaments that when shortened result in contraction of the individual skeletal muscle fiber (Oregon). The process through which skeletal muscle is 'converted' into meat is known as rigor mortis. Rigor mortis sets in when the energy reserves (ATP) of the muscle fibers are depleted so any contractile potential is lost, thus preventing shortening of the sarcomeres. Shortening of the sarcomeres decreases the tenderness of the meat since it causes the proteins that compose it to overlap (Locker and Hagyard, 1963; Marsh and Leet, 1966). For this reason, electrical stimulation (ES) is applied to the animal post exsanguination in order to accelerate the depletion of ATP in an attempt to improve meat palatability (Adeyami and Sazili, 2014). Hastening the onset of rigor mortis through ES is desirable from a quality standpoint because if the carcasses are chilled with significant ATP and glycogen reserves, then the rapid decrease in temperature (below 15°C) will cause what is known as 'cold shortening' (Savell et al., 2005). The presence of ATP allows for the continuation of contraction (shortening) of the sarcomeres that, as previously outlined, results in a decrease in tenderness. Lower temperatures post-harvesting help protect against bacterial growth, but they promote further contraction of the ATP-rich muscle fibers. Therefore, depletion of ATP through conversion of glycogen to lactic acid (lower pH) prevents cold shortening. This prevents a decrease in tenderness in the meat. Another interaction

in what is known as the calpain-calpastatin system is affected by postmortem ES. Calpain is an enzymatic protease that contributes to proteolytic activity in muscle whereas calpastatin is an inhibitor of calpain. (Melloni et al., 1992). Applying ES postmortem is considered to stimulate calpain through release of calcium ions that contribute to increased calpain activity (protein degradation) (Adeyami and Sazili, 2014). There is an opposing view on this position that states that ES negatively affects calpain activity due to rapid decline in pH, which is correlated with a decrease in activity of calpain and increases the activity of calpastatin (Maddock-Carlin et al., 2006). Nonetheless, the prevailing understanding in the industry is that ES benefits meat quality by prevention of cold shortening and its contribution to calpain activity through calcium ion release.

Conclusion

Maintaining efficient beef production in order to meet growing demand is becoming more critical and challenging for producers. Changing consumer preferences that are not in align with conventional practices are forcing producers to search for alternatives that safeguards both the efficiency of their operations and the marketability of their cattle. For this reason, our first research study focused on investigating the differences in feed and growth efficiency between a probiotic blend and monensin in order to evaluate the potential for this blend to serve as an alternative for producers.

Our second research study focused on analyzing these interacting effects of HS and ZH supplementation by focusing on parameters such as growth performance, respiration rate, rectal temperature, and carcass composition. With projections of increasing global temperatures and a higher susceptibility to heat stress in cattle due to selection of highly productive cattle makes combating the negative effects of heat stress of utmost importance; however, the potential

interaction effects of heat stress with the crucial growth enhancement technology, ZH, that plays its own role in promoting production efficiency brings on another challenge.

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APPENDIX A: Evaluation of Performance effects from supplementation of Probiotic (MULTSACCH[®]) and Monensin (Rumensin) in Feedlot cattle

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Abstract

Thirty-two Hereford x Simmental steers (250.0 ± 15 kg **Initial BW**) were assigned to four dietary treatments in a completely randomized design (4 steers/pen, 2 pens/treatment) in order to evaluate the effects of supplementing Monensin (**MON**; Rumensin; Elanco Animal Health, Greenfield, IN) in comparison and in combination (**CMB**) with a proprietary Probiotic blend (**PRO**; MultSacch®, Biomart Nutrição Animal Imp. and Exp. Ltda.) on the growth performance, feed efficiency, and on cost of gain. Individual feed intake was monitored using a GrowSafe System (GrowSafe Systems Ltd. Calgary, AB, Canada) and weights were recorded weekly for a 90 d growth data period. Supplementation with PRO and CMB both increased Gain:Feed (G:F) by 6% with respect to control (**CON**) steers ($P = 0.01$). No treatment effects were detected in initial body weight (IBW), final body weight (FBW), or average daily gain (ADG) ($P > 0.05$). DMI was lowest in CMB steers ($P = 0.04$), and this same group had the lowest cost of gain ($P < 0.001$). Present findings indicate that when compared with MON, under these experimental conditions PRO seems to be just as effective in stabilizing intraruminal milieu and increasing ruminal fiber utilization when steers are fed rapidly fermentable carbohydrate grain-based diets, thus improving feed efficiency without the need of MON supplementation. However, combined use of PRO and MON results in the lowest cost of gain ($P = 0.03$). Further experimentation is required to ascertain the effect substituting MON with different mixtures of Probiotics will have long term on cattle growth performance and profitability.

Introduction

Ruminants in production settings utilize several different types of feedstuffs in order to meet growth performance expectations. In order to properly digest these feedstuffs that are vital for growth, a healthy and thriving ruminal ecosystem must be maintained (Ghazanfar et al., 2017). Two options producers have for enhancing the ruminal ecosystem are ionophores and yeast based probiotics (YP).

Monensin (MON) is the most widely used ionophore among cattle feeders in the United States (Schelling, 1984). Monensin is classified as a polyether antibiotic; however, MON should not to be confused with therapeutic antibiotics because it does not ‘kill’ bacteria, rather bacteria, more specifically gram-positive bacteria, are targeted in order to reduce lactic acid production and methanogenesis in the rumen and increase molar proportion of propionate and N retention (Russell and Strobel, 1989). In addition to helping reduce waste products, MON fed cattle have lower incidence of bloat, ruminal acidosis, and incidence of coccidiosis (Stock et al., 1995).

Supplementation of YPs has been considered to be an effective way to stabilize intraruminal milieu and fermentation in ruminants fed high-concentrate diets, increase nutrient digestibility and overall animal performance (e.g. feed efficiency and weight gain) (Silva et al., 2018; Chaucheyras-Durand et al., 2012). One species of yeast commonly incorporated in ruminant diets and in Probiotic blends is *Saccharomyces cerevisiae* (SC). *Saccharomyces cerevisiae* live cells have been reported to have the ability to remove oxygen from the rumen environment and release essential enzymes, vitamins and other nutrients or growth factors, which could facilitate bacteria to have a high viability and activity in the rumen (Ghazanfar et al., 2017; Newbold et al., 1993). Despite these known positive effects of YP supplementation in ruminants, MON remains the most common feed additive used around the globe to enhance feed efficiency and overall performance (Silva et al., 2018).

Based on the similarity in modulation of ruminal fermentation between MON and YPs, we hypothesize that combined supplementation of PRO and MON will result in a compounding of positive effects on ruminal fermentation that will translate into improved growth performance traits.

Experimental Design and Methods

The protocol for this study was reviewed and approved by the University of Arizona Institutional Animal Care and Use Committee. A cattle feeding trial was conducted using 32 crossbred Hereford x Simmental steers (250 ± 15 kg **Initial body weight**) in order to evaluate the effects supplementation of a Probiotic blend with / without Monensin has on growth performance effects in growing steers. Steers were assigned to four dietary treatments in a completely randomized design (4 steers/pen, 2 pens/treatment). Steers received an experimental diet with the following treatments: (1) no additives (CRT); (2) Monensin (MON) supplementation (Rumensin Elanco Animal Health Indiana USA, 20% commercial premix) (1,412.7 mg Monensin Sodium / Supplement kg; fed at 2.14% of weight of delivered feed); (3) Probiotic additive brand MULTSACCH®, (PRO) composed of *Bacillus subtilis* (minimum of 3×10^9 CFU / g), *Bifidobacterium bifidum* (minimum of 1×10^9 CFU / g), *Enterococcus faecium* (minimum of 1×10^9 CFU / g), *Lactobacillus acidophilus* (minimum of 1×10^9 CFU / minimum), *Lactobacillus buchneri* (minimum of 2×10^9 CFU / g), *Lactobacillus casei* (minimum of 1×10^9 CFU / g), *Lactobacillus lactis* (minimum of 1×10^9 CFU / g), *Saccharomyces cerevisiae* (minimum of 2×10^8 CFU / g) manufactured by Biomart Animal Nutrition Import and Export. Ltd - Martinópolis / SP - Brazil (1 g per 100 kg of live weight) and (4) Combination (CMB) of MON and PRO. Steers were fed an 88% concentrate diet mostly composed of ground-corn and alfalfa hay formulated to meet or exceed National Research Council recommendations. The

ingredient and nutrient composition of the mixed concentrate is presented in Table 1. Steers were fed once per day and supplements were added at feeding by top dressing feed then mixing thoroughly with handheld drill based auger. Individual feed intake was monitored using a GrowSafe System (GrowSafe Systems Ltd. Calgary, AB, Canada). Water was offered *ad libitum*. The growth performance trial lasted for 97 days, including a 7-day adaptation period and 90-day growth data collection period. Steers were weighed weekly throughout the 90-day period. The average daily gain (ADG) and dry matter intake (DMI) were determined and feed efficiency was calculated.

Statistical Analysis

A completely randomized experimental design was utilized. Data were analyzed cumulatively using the MIXED procedure of SAS (SAS 9.3, SAS Institute Inc., Cary, NC, USA). The LSMEANS option was used to calculate all comparison estimates for the fixed effects of treatment groups. Fixed variables included treatment. Significance was declared at $P < 0.05$. When significant effects were observed, differences between the means were evaluated.

Results

Table 2. summarizes the performance values for all experimental groups. Steers in the CON group had ADG, DMI, and G:F values comparable to facility values using the GrowSafe system. Supplementation with PRO and CMB both increased G:F by 6% with respect to CON steers ($P = 0.01$). No treatment effects were detected in ADG, IBW, or FBW ($P > 0.05$). Dry Matter Intake and cost of gain were lowest in CMB steers ($P = 0.04$ and $P < 0.001$, respectively). The PRO group was the second most profitable group in this trial based on cost of gain, and there were no significant differences in cost of gain between MON and CON steers. Present

findings indicate that when compared with MON, PRO supplementation seems to be more effective in improving growth performance characteristics.

Discussion & Implications

Feed supplements play a crucial role in improving growth and feed efficiency in beef cattle. As previously mentioned, MON and YP supplementation both contribute to improvements in rumen health, thus resulting in greater overall animal performance. These analogous benefits to rumen function and overall productivity make PRO supplementation an attractive option for cattle feeders to incorporate into their feed formulations. Combined use of PRO and MON in this experiment resulted in the lowest DMI and the lowest cost of gain. This boosted performance can potentially be a result of a compounding of the complementary benefits that both of these additives provide to the rumen environment, which is what dictates feed efficiency.

It is well documented that the efficacy of YPs, alone or in combination with MON, varies at different types and doses of live yeast cells, levels of concentrate in the diet, and age of the animal (Wagner et al., 2017). High concentrate diets, like the one used in this experiment, are often associated with a lowering of the ruminal pH, which is linked to negative effects on growth performance in afflicted cattle (Jiao et al., 2017). Jiao et al. (2017) further reports that diets supplemented with SC result in an increase in anaerobic bacteria that increase fiber digestion and flow of microbial protein from the rumen and that yeasts stabilize rumen pH by reduction of lactic acid producers and stimulation of lactic acid utilizers, thus alleviating incidence of subacute rumen acidosis. In this trial where a high concentrate diet was fed to the steers for 97 d, it is expected that the ruminal pH of the steers was altered due to the significant inclusion of grain in the diets. Future experimentation should consider measuring ruminal pH levels in order

to correlate the differences in feed efficiency with the counteracting effects PRO and MON have on the ruminal disturbance caused by the high grain diet.

A trial of similar length and experimental diet content but with a different probiotic blend observed no added benefits in growth performance parameters through combined supplementation of YP and MON (Pancini et al., 2020). This difference can potentially be attributed to the use of different YP blends and the fact that the cattle in the trial Pancini et al. (2020) conducted were in a later stage of production compared to the steers in our study (IBW = 446 vs IBW = 250 kg).

The improvement of feed efficiency observed through PRO supplementation in this experiment with respect to the CON group is comparable to an improvement in feed efficiency noted in a meta-analysis where the results of 18 experiments involving YP supplementation in beef cattle performance were examined (Wagner et al., 2016). However, this meta-analysis noted a correlation between an increase in DMI and ADG through YP supplementation, which differs from the results in this experiment where no significant difference in ADG or feed intake was observed between the experimental groups. This inconsistency can be explained if the days on feed (DOF) is taken into consideration. Wagner et al. (2016) noted the most improvement in DMI occurred in experiments where cattle were supplemented YPs for 200 d vs 100 d. The current study supplemented YPs for 90 d. Therefore, the DOF appears to have a significant impact on the effectiveness of the YP blend in stabilizing intraruminal milieu and increasing ruminal fiber utilization when steers are fed rapidly fermentable carbohydrate grain-based diets.

With regard to MON vs PRO supplementation, the results from this experiment differ from a previous experiment where YP supplementation lowered ADG and had no effect on feed efficiency, cost of gain, or DMI (Swyers et al., 2014). Swyers et al. (2014) attributed these

effects on the composition of the finishing ration fed to the cattle. The finishing ration consisted of 19.7 % dry distillers grains and solubles, which resulted in a lower component of grain in the ration (63%). The ration used in our trial had 73% grain, which is closer in line with the recommended range (70 – 85 %) Swyers et al. (2014) noted in their discussion. Thus, the difference in results can be attributed to differing levels of grain in the rations fed to the cattle.

Under our experimental conditions, YP supplements have the potential to improve growth performance characteristics when used in combination with MON; however, any decision to consider YPs as an alternative for MON must take into consideration the added benefits of MON supplementation that are not observed in YP supplementation, such as the reduction in coccidiosis incidences. Further experimentation is required in order to understand the growth performance effects of YP supplementation in beef cattle.

Table 1.0 Ingredients and nutritional composition of diets

Item		Experimental Diet¹
Ingredients	% of DM	
Alfalfa (ground)		13.7
Corn (cracked)		73.2
Mineral Mix²		2.1
Molasses		6.3
Soybean Meal		3.8
Urea		0.9
Nutritional Composition		
% Total moisture		12.74
% Ash		6.34
%NDF		12.14
%ADF		6.37
% CP		14.2
% Nitrogen		2.28
TDN (na)		77

Table 2.0 Performance and cost effects of with/without PRO and MON supplementation

Item	Treatment				SEM	P-value
	CON	MON	PRO	CMB		
Initial body weight, kg	241.3	246.7	242.5	242.0	6.32	0.93
Final body weight, kg	382.1	391.5	375.5	378.5	12.43	0.82
ADG, kg	1.3	1.4	1.4	1.3	0.04	0.28
DMI, kg/d	8.3 ^b	8.2 ^b	8.0 ^{ab}	7.6 ^a	0.18	0.04
G:F	0.16 ^a	0.165 ^{ab}	0.17 ^b	0.17 ^b	0.002	0.01
Cost of feed/d, \$	0.45	0.47	0.46	0.44	-	-
Cost of gain, \$/kg¹	2.8 ^c	2.8 ^c	2.7 ^b	2.6 ^a	0.03	< 0.0001

¹(Cost of feed/d) / (G:F)

Conflict of Interest

The authors declare no conflict of interest.

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APPENDIX B: Detecting performance effects and body composition effects of β -agonist supplementation under heat stress conditions in Red angus steers

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Abstract

Red Angus steers (n=24; 260 ± 25 kg) were used to analyze the effects of supplementation of zilpaterol hydrochloride (ZH) under heat stress conditions on respiration rate (RR), rectal temperature (RT), growth performance (GP), Biological Impedance Analysis (BIA), carcass traits (CT), and complete blood count (CBC). Steers were randomly assigned to a 2 x 2 factorial treatment arrangement (n=6/group) with factors including heat stress (HS; Temperature Humidity Index (THI) = 73 to 85) or thermal neutral (TN; THI = 68) conditions and with/without supplementation of ZH (0 or 8.38 mg/kg/d on 88% DM basis). Steers were provided 9 d to acclimate to tie stalls rooms under TN conditions before starting the study. TN steers were pair-fed to the average daily dry matter intake (DMI) of HS steers. *Ad libitum* water intake (WI) was recorded daily. HS and TN steers were harvested on d 22 and 23, respectively. By design, DMI did not differ between environment or supplement groups ($P>0.31$). Initial body weight did not differ between environments or supplement groups ($P>0.62$). RT, RR, and WI were greater ($P<0.01$) in HS steers compared to TN steers. There was a supplement by environment interaction ($P=0.02$) for RT, as HS steers fed ZH had lower RT than HS control steers (39.1 vs 39.5 °C, respectively). Average Daily Gain (ADG) was 20% higher ($P=0.04$) in HS steers compared to TN steers. BIA phase angle (PA), resistance (R), and reactance (R_c) values were significantly lower in HS steers by 19%, 8.5%, and 25%, respectively ($P \leq 0.04$). CT and CBC did not differ due to environment, treatment, or environment by treatment interactions ($P>0.05$). Our results suggest that feedlot steers under our experimental conditions display some sensitivity to HS through GP, RR, RT, and BIA; however, this does not translate to a significant impact on CT or CBC. Furthermore, ZH supplementation did not appear to impact animal well being negatively.

Keywords: heat stress, β -adrenergic agonists, feedlot

Introduction

Heat stress is a serious hindrance to livestock performance. Livestock enter a state of heat stress when their core body temperature exceeds the normal specified range and when their total heat load exceeds the rate at which they are dissipating heat (Bernabucci et al., 2010). This results in an antagonism where several adjustments are made in behavior, physiology, and metabolism that alleviate hyperthermia but have negative consequences on performance (Bernabucci et al., 2010).

Livestock producers implement a range of technologies in order to optimize animal performance. One of these technologies, used extensively in the cattle feeding industry, is Zilpaterol hydrochloride (ZH), a Beta-adrenergic agonist (BAA). BAAs such as ZH bind to G-protein coupled receptors on fat and muscle cell surfaces and stimulate lipolysis and protein synthesis while inhibiting lipogenesis and proteolysis (Strydom et al., 2009). This has been reported to increase overall leanness, and thus cutability, of beef carcasses without increasing feed intake; however, ZH supplementation appears to negatively affect quality, specifically by reducing marbling and tenderness score (Vasconcelos et al., 2008; Avendaño-Reyes et al., 2006). Zilpaterol hydrochloride was commonly used in the United States feedlot industry between 2006 and 2013 until it was removed from the market by the manufacturer. Anecdotal accounts indicated that cattle fed ZH had higher levels of stress, mortality rate, and lameness upon arrival at the packing plants, particularly during the summer months.

The adrenergic system that is stimulated in order to increase muscle accretion in ZH supplemented cattle is also stimulated when cattle become heat stressed (Swanson et al., 2019). Therefore, the objective of this study was to determine the effects simultaneous stimulation of

the adrenergic system, through supplementation of ZH and exposure to heat stress conditions, has on animal well-being, RR, RT, growth performance (GP), carcass traits (CT), and body composition. We postulate that over stimulating the adrenergic receptors will increase stress in cattle and therefore the experimental group is expected to significantly under perform in the parameters being measured compared to the control group.

Materials & Methods

Animals and Experimental Design

The protocol for this study was reviewed and approved by the University of Arizona Institutional Animal Care and Use Committee. A cattle feeding trial was conducted using 24 red Angus steers in order to evaluate the effects of feeding ZH in combination with and without heat stress on growth performance, non-performance characteristics, Biological Impedance Analysis (BIA), and carcass traits. Red Angus steers were transported from a commercial operation in Nebraska to the University of Arizona Feedlot (Tucson, AZ). Upon arrival, steers were fed an 88% concentrate diet mostly composed of ground-corn and alfalfa hay formulated to meet or exceed National Research Council recommendations, which was the same ration fed throughout the study. Twenty-four steers (260 ± 25 kg) were then chosen for the study and transported to the University of Arizona's Agricultural Research Complex (ARC). These steers were randomly assigned to a 2 x 2 factorial treatment arrangement ($n = 6$ / group) with factors including heat stress (HS; Temperature Humidity Index (THI) = 73 to 85) or thermal neutral (TN; THI = 68) conditions and with / without supplementation of ZH (0 or 8.38 mg/kg/d on 88% DM basis). All steers were provided 7 d to acclimate to tie stalls rooms in environmental chambers under TN conditions before starting the study. TN steers were pair-fed to the average daily dry matter

intake (DMI) of HS steers. This means TN steers were only fed as much as the HS steers consumed the previous day in relation to their body weight. *Ad libitum* water intake (WI), respiration rate (RR) and rectal temperature (RT) were recorded daily. RR was measured by counting flank movements for 60 s. RT were measured using standard digital thermometers. Average daily WI, F:G, and ADG were calculated at the end of the experimental trial.

Biological Impedance Analysis

Biological Impedance Analysis was performed at d -2 and 22 (live animal) and on the hot carcass at harvesting (d 23 and 24). A four-terminal Quantum V (RJL Systems, Detroit, MI) was used to measure reactance (X_c), resistance (R_s), and phase angle (PA). Two sets of spaced electrode terminals were used in order to transmit an electrical current across tissues. Electrical current was then applied for 30 s and measurements were recorded in 5 s intervals and were then averaged and used for analysis.

Carcass Data Collection

All cattle were harvested in the abattoir of the University of Arizona Food Product and Safety Laboratory. HS and TN steers were harvested on d 22 and 23, respectively, after having been provided with the assigned morning feed. Hot carcass weight (HCW) was collected and carcasses were chilled for 7 d. After chilling, carcasses were ribbed at the 12th rib, and USDA quality and yield traits were measured (USDA, 1997). Carcass traits measured included cold carcass weight (CCW), longissimus muscle area, kidney pelvic and heart percentage, and marbling score.

Environmental Temperature Recordings

Figure 1 displays the cyclical daily THI steers were exposed to after the acclimation period. THI values were calculated using the mean temperatures and relative humidity

percentage values obtained from the data logger (EasyLog USB, Lascar Electronics Inc., Erie, PA). THI was defined by the formula (Australian Livestock Export Corporation, 2020):

$$THI = 0.8 \times T_{db} + RH \times (T_{db} - 14.4) + 46.4$$

Where: T_{db} = dry bulb temperature °C and RH = relative humidity percentage.

TN steers were exposed to a constant THI of 68 (no stress) whereas HS steers were exposed to THI values that ranged from a minimum of 73 (mild stress) and a maximum of 85 (severe stress).

CBC

Blood was collected via jugular venipuncture on d 7, 14, and 21 of the experiment. Blood samples were then sent to a laboratory for a complete blood count analysis (University Pet Clinic, Tucson, AZ).

Statistical Analysis

A completely randomized experimental design was utilized with a 2 x 2 factorial treatment arrangement. Data were analyzed using the MIXED procedure of SAS (SAS 9.3, SAS Institute Inc., Cary, NC, USA). The LSMEANS option was used to calculate all comparison estimates for the fixed effects of environment (ENV), treatment (TRT), and ENV x TRT interaction. Variables included treatment and environment and their respective interactions. Tendencies were declared ($0.05 > P < 0.1$). Significance was declared at ($P < 0.05$). When significant effects were observed, differences between the means were evaluated.

Results & Discussion

Performance data

Live performance data for the treatment period are displayed in Table 1. By design, no effects were detected for DMI for TRT, ENV, and TRT x ENV ($P \geq 0.29$). No differences were

detected for F:G between TRT and ENV x TRT ($P \geq 0.91$), however, there was a tendency in ENV where HS steers had a F:G that was 17% less than TN steers ($P = 0.06$). ADG did not differ significantly between TRT or ENV x TRT groups ($P \geq 0.37$) but there was an effect detected for ENV where HS steers had an ADG 18% higher than TN steers ($P = 0.03$). Similarly, there was no effect detected for WI in TRT or ENV x TRT groups, but there was an effect detected between ENV groups where HS steers on average consumed 37% more water than TN steers ($P = 0.003$).

Supplementation of ZH has consistently demonstrated to be an effective way to improve ADG and F:G (Rathmann et al., 2012). Several large-pen steer studies have demonstrated that ZH supplementation can improve ADG by 13-15% and G:F by 16-18% (Elam et al., 2009; Montgomery et al.). A large-pen study in Nebraska looking at the effects of ZH supplementation between steers in shaded pens and steers in open pens found no difference in ADG between treatment groups; however, they did note that cattle fed in open pens tended to have a greater ADG than cattle fed in shaded pens ($P = 0.10$) (Boyd et al., 2015). When considering these data and the results of the current study, a possible justification for the increased ADG noted in the HS steers in the current study and the tendency Boyd et al. (2015) noted in the heat stressed cattle could be explained through increases in water consumption. Cattle that are exposed to HS drink more water in order to maintain normal body temperature and this increases the overall weight of the animal (1 liter of water is equal to 1 kilogram). Furthermore, differences in ADG would be expected to translate into differences in carcass weight, which is not the case in this study. Therefore, it is safe to postulate that the differences noted in ADG and F:G are due to water weight and not muscle growth.

Non-performance data

The data collected for RT and RR in the study are presented in Table 1. Mean values for ENV were as expected with RR and RT being higher in HS steers than TN steers ($P < 0.0001$). There was a supplement by environment interaction ($P = 0.02$) for RT, as HS steers fed ZH had lower RT than HS control steers (39.1 vs 39.5 °C) and these RT values did not differ significantly with either TN CON steers or TN ZH steers. Steers supplemented ZH tended to have lower RR than CON steers in the same environments (98.9 breaths/min vs 104.7 breaths/min in HS; 45.4 breaths/min vs 52.6 breaths/min in TN) ($P = 0.06$). The differences noted between environments is consistent with the results from a trial where growing cattle exposed to TN conditions displayed lower RR and RT values than the HS cohort (O'Brien et al., 2010). Boyd et al. (2015) noted similar results in ZH supplemented steers having lower body temperatures than CON steers. The tendency noted in this experiment for RR to be lower in ZH supplemented steers versus CON steers in the same environment is not consistent with results from the previously mentioned trial conducted by Boyd et al. (2015) and another trial conducted by Hales et al. (2014) that both noted numerically higher RR in ZH supplemented steers although in this latter trial the differences were not significant. The results from this experiment with regard to lower RT and RR contradict the notion that BAA supplementation under HS conditions exacerbates the heat load on an animal.

Carcass data

Data collected on carcasses are presented in Table 1. No effects were noted in ENV, TRT, and ENV x TRT for hot carcass weight (HCW), cold carcass weight (CCW), kidney, pelvic, heart fat percentage (KPH), 12th rib fat thickness (FT), marbling score (MS), and longissimus muscle area (LM) ($P > 0.05$). Two tendencies were observed in MS and LM values.

LM areas of HS steers were 5% greater than LM areas of TN steers ($P = 0.07$) and MS tended to be 17% greater among ZH supplemented steers than CON steers ($P = 0.06$). A tendency for greater LM areas in HS steers has not been observed in any study measuring carcass characteristics. The tendency for MS to be higher in ZH supplemented cattle is contradictory to the data from several studies where ZH supplementation has been observed to in fact reduce MS (Vasconcelos et al., 2008; Montgomery et al., 2009; Boyd et al. 2015; Rathman et al., 2012). Meeting growth requirements is necessary for fat deposition and given that ADG was numerically higher (2.11 kg/d vs 2.07 kg/d) in ZH supplemented cattle compared to CON supplemented cattle, it is possible that this higher rate of gain is what allowed ZH steers to have a tendency for a higher MS.

Biological Impedance Analysis data

The values determined in the BIA are presented in Table 2. For live animal measurements there was no significant effects for TRT or TRT*ENV; however, there was an ENV effect where phase angle (PA), resistance (R), and reactance (R_c) values were significantly lower in HS steers by 19%, 8.5%, and 25%, respectively ($P \leq 0.04$). Postmortem values did not display any ENV*TRT effect but ENV and TRT differences tended to be lowest in PA, R, and R_c for the HS CON group versus all other groups by 52%, 34%, 56%, respectively ($1.0 \geq P \geq 0.06$).

Phase angle is the arc tangent value of the ratio of reactance versus resistance and it is therefore the primary value used to analyze body composition through BIA. In humans, lower phase angle values have been determined to be correlated with increased incidence of disease states and as clinical status improves, PA increases (Kumar et al., 2012). The PA values from the live animals in this study are therefore consistent with the current understanding that ruminants

are negatively affected when they are exposed to hyperthermic conditions; furthermore, there was no difference in supplementation of ZH noted in the BIA values as is consistent with Gibbs et al. (2019) and Swanson et al. (2019). The low PA values recorded postmortem indicate that ZH supplemented steers are less likely to display cellular damage than CON supplemented steers; however, this discrepancy between values recorded in live animals and postmortem brings into question the accuracy of BIA in 'hot' carcasses because the extracellular fluid, which is drained during harvesting through exsanguination, is what offers electrical resistance to the electrical current being applied (Kumar et al., 2012). Therefore, performing BIA under these starkly different conditions will likely result in inconsistent results, as is demonstrated in this experiment. Further studies recording BIA values in live animals and postmortem are required in order to solidify BIA as a reliable estimator of body composition and animal well being.

Complete blood count data

Results from the laboratory analysis were analyzed in reference to the Hematologic Reference Ranges established in the Merck Veterinary Manual (Fielder, 2015). All values were within normal ranges. There were no significant interactions ($P > 0.05$) in white blood cell, basophil, monocyte, eosinophil, lymphocyte, and neutrophil concentrations between ENV, TRT, or ENV*TRT.

Discussion and Implications

Literature evaluating the interacting effects of ZH supplementation and exposure to heat stress conditions is limited. Results of this study suggest that feedlot steers under our experimental conditions display some sensitivity to HS through GP, RR, RT, and BIA; however, this did not translate to an impact on CT, CBC, or overall animal well being. Furthermore, ZH supplementation under HS conditions appears to impact thermoregulatory responses positively,

yet this did not impact GP or CT. Two important variables to take into account when evaluating the results of this experiment are the breed and weight of the cattle under experimentation. The Angus breed, and *Bos taurus* cattle in general, are considered to be less tolerant to heat stress and this is frequently attributed to hide color; however, this experiment was conducted indoors and no solar radiation was applied to the steers so any dissipation of heat, or the lack thereof, must be due to hair density and hair length (Bernabucci et al., 2010). Performing this experiment with *Bos indicus* cattle or other *Bos taurus* cattle such as the Senepol breed that express the ‘slick’ gene can ascertain this point. Furthermore, the initial body weight of the steers used in this experiment (260 ± 25 kg) is significantly lower than the normal weight range of cattle when ZH is supplemented in the different experiments cited and in most commercial operations. Cattle at different stages of production partition nutrients differently, with the deposition of fat being ranked lowest in physiological importance and muscle higher (Byers et al., 1998). Therefore, it is possible that this experiment is lacking in its ability to detect ZH induced changes in CT and BIA since cattle at this stage of production already have an increased priority placed on lean muscle deposition. Further studies on cattle at different stages of production can help determine the true effects ZH supplementation and HS conditions have on CT.

Determining the effects of ZH supplementation under different environmental conditions can guide producers’ judgment when determining BAA supplementation at different times of the year and dependent on the type of cattle being fed. Further experimentation is needed to determine the effect simultaneous stimulation of the adrenergic system through ZH supplementation and heat stress exposure has on GP, RR, RT, BIA, CT, and CBC.

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Table 3 Performance and characteristic values

Item	Thermal				SEM	P-value		
	Neutral		Heat Stress			ENV	TRT	ENV*T RT
	CON	ZH	CON	ZH				
Initial BW, kg	254.7	261.3	262.3	262.4	9.2	0.64	0.72	0.72
Final BW, kg	299.7	303.2	312.7	317.5	10.0	0.19	0.68	0.94
DMI, kg/d	6.8	7.2	7.1	7.3	0.29	0.50	0.29	0.90
ADG, kg	1.95 ^a	1.82 ^a	2.19 ^b	2.40 ^b	0.18	0.03	0.84	0.37
F:G	3.5	4.0	3.2	3.0	0.14	0.06	0.96	0.91
Water intake, (L/d)	21.8 ^a	23.0 ^a	34.47 ^b	35.3 ^b	3.64	0.003	0.79	0.96
Respiratory rate, breaths / min	52.6 ^a	45.4 ^a	104.7 ^b	98.9 ^b	3.16	<0.0001	0.06	0.83
Rectal temperature, °C	^A 38.5 ^a	^A 38.6 ^a	^{AC} 39.5 ^b	^{AB} 39.1 ^b	0.09	<0.0001	0.13	0.02

^{a-b}Differences between means were evaluated within rows at $P < 0.05$ for ENV effects.

^{A-C}Differences between means were evaluated within rows at $P < 0.05$ for ENV*TRT effects.

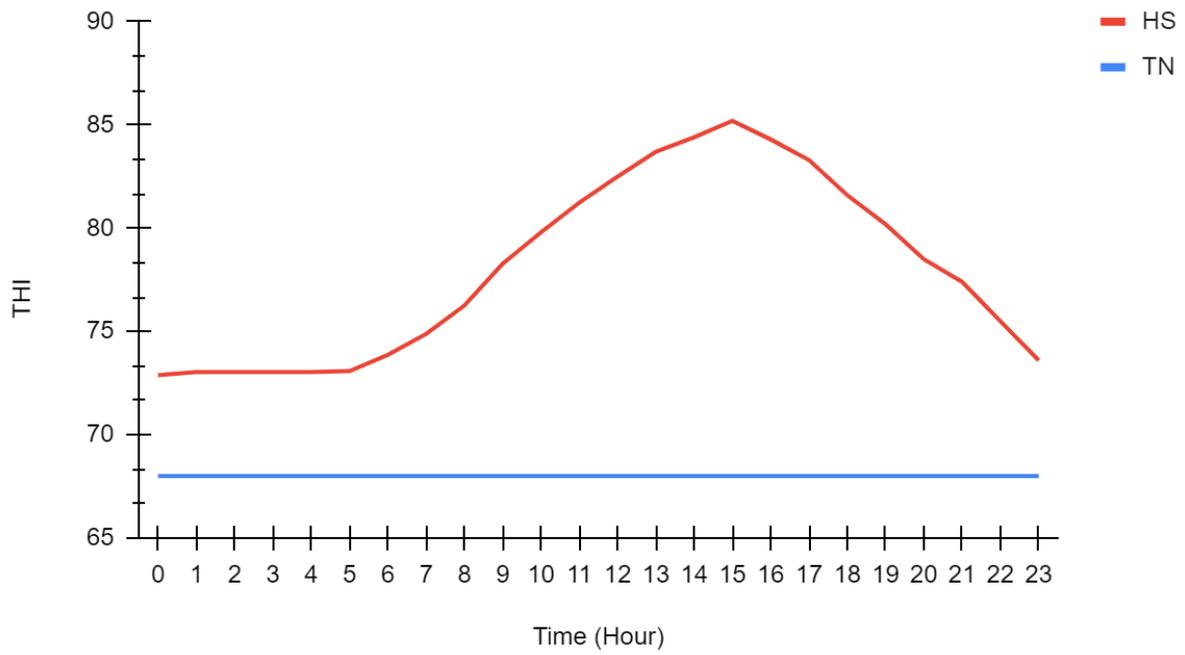
Figure 1. Diurnal Cycle TN vs HS**Figure 1 Durnal cycle TN vs HS**

Table 4 Carcass traits and BIA values

Item	Thermal Neutral		Heat Stress		SEM	P-value		
	CON	ZH	CON	ZH		ENV	TRT	ENV*TRT
<i>Carcass Traits</i>								
HCW, kg	152.9	159.1	159.8	159.4	5.86	0.54	0.63	0.58
CCW, kg	149.2	151.5	153.5	153.9	5.82	0.57	0.82	0.87
KPH, %	1.0	1.0	1.0	1.0	0.0004	0.20	0.20	0.20
12th Rib fat, cm	0.11	0.13	0.11	0.12	0.012	0.46	0.20	0.54
LM area, cm ²	57.9	55.0	58.9	59.7	1.49	0.07	0.49	0.24
Marbling score ¹	300.0	350.0	300.0	333.3	21.10	0.70	0.06	0.70
Carcass Gain / Total DMI								
<i>BIA Live Animal</i>								
Resistance	18.1 ^b	18.35 ^b	16.91 ^a	16.59 ^a	0.7	0.04	0.97	0.69
Reactance	3.04 ^{bc}	3.09 ^c	2.41 ^{ab}	2.16 ^a	0.24	0.004	0.68	0.56
Phase Angle	9.46 ^{ab}	9.63 ^b	7.88 ^{ab}	7.6 ^a	0.62	0.01	0.94	0.72
<i>BIA Postmortem</i>								
Resistance	52.59 ^b	54.66 ^b	35.58 ^a	54.65 ^b	5.47	0.12	0.09	0.14
Reactance	27.84 ^b	29.65 ^b	12.68 ^a	28.9 ^b	5.41	0.19	0.09	0.17
Phase Angle	26.6 ^b	28.7 ^b	13.21 ^a	27.44 ^b	4.06	0.09	0.06	0.16

¹300= Slight, 400= Small (USDA, 1997)

^{a-b}Differences between means were evaluated within rows at ($P < 0.05$).

Figure 2. Phase Angle - Live animal vs Postmortem

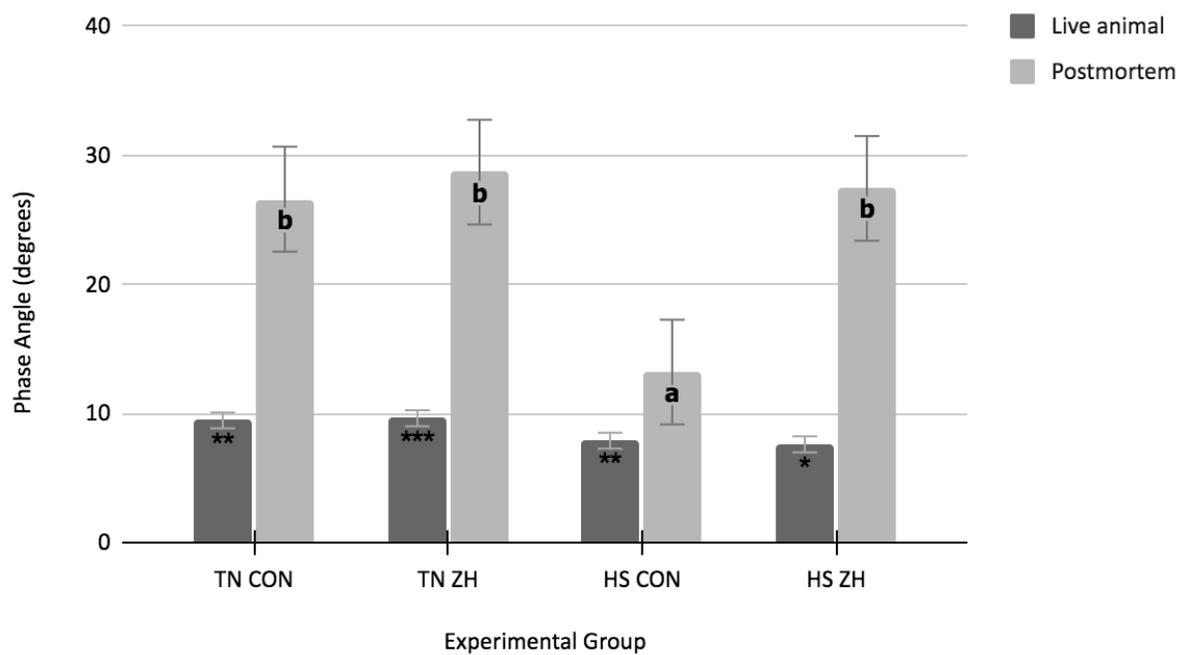


Figure 2 Phase Angle - Live animal vs Postmortem